Jacob, Rebecca (ASRC)

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Sent: Wednesday, December 04, 2002 10:58 AM
To: Jacob, Rebecca (ASRC)
Subject: FW: Articles

-----Original Message-----
Fr m: Solomon, Terrance
Sent: Wednesday, December 04, 2002 10:57 AM
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Cc: DeSanto, Matthew
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The following articles **were not** available full-text using STIC resources:

Retina
Vol-18
Issue-1
" Pharmacologic Vitreolysis"
Sebag, J.
Pages:1-3
Year-1998

For **Matthew F. DeSanto, AU 3763, 305-3292, US serial NOT GIVEN**.

Thanks,

Terry Solomon
EIC 3700
305-5932
CP2, 2C08

Editorial

Pharmacologic Vitreolysis

The annals of surgery reflect numerous instances wherein widely practiced treatment modalities were replaced over time by less invasive and, eventually, atraumatic interventions. Such evolution derives primarily from increased knowledge of the basic pathogenic mechanisms of disease. Often, there is substitution of surgical therapies with medical treatment modalities and, ultimately, with approaches aimed at the prevention of the disease entirely. Consider, for example, that abscesses, once routinely treated by surgical drainage, became amenable to antibiotic regimens that are not only less traumatic and more successful, but considerably less expensive. Improved sanitary conditions and vaccines subsequently eradicated many infectious diseases that once plagued entire societies and influenced the course of history. This very type of evolution is just beginning to move beyond surgery in the area of vitreoretinal diseases.

Vitreous is an extracellular matrix that fills the center of the eye with a clear viscoelastic tissue that maintains clarity and protects against the untoward effects of eye, head, and body movements.[1] Composed 98% of water, the corpus vitreus exists in a gel state (Figure 1) because of the intricate organization of its macromolecular components. Collagen and hyaluronan are the macromolecules of import, but a number of proteoglycans, glycoproteins, and other lesser molecules may play a critical role in the organization of the two major macromolecules (Figure 2) into a three-dimensional structure[2,3] that achieves the aforementioned functions of clarity and shock absorption.[1,4] These intermediary molecules may or may not be the same as those responsible for the adherence of the posterior vitreous cortex to the internal limiting lamina of the retina.[5] This information is critical in properly designing approaches to alter vitreous on a molecular level. If the gel state and vitreo–retinal adhesion are both the result of one or a group of closely related molecule(s), then one agent will probably suffice to induce salubrious alterations in vitreous structure. If, as is likely, these two properties of vitreous are the consequences of two disparate molecules or groups of molecules, then a single agent will not suffice if there is to be any specificity to the action of the agent. In such a case, more than one agent will be needed to alter the molecular state of the corpus vitreus properly, as well as the vitreo–retinal interface.

Whereas vitreous has long been overlooked as crucial in the pathophysiology of various blinding disorders, this has begun to change in light of recent significant advances in knowledge of the structure, function, and pathobiology of this unique matrix.[4] Advances have led to the development of an impressive array of surgical instruments, techniques, and results for conditions that permanently blinded many people just a generation or two ago. Success has engendered complacency with respect to the traumatic nature of surgery, its inherent limitations, and the enormous costs to society. As a result, involved parties have overlooked the fact that as successful as a surgical approach may seem, it can never be as beneficial as a nonsurgical treatment, both in terms of clinical outcome measures and socioeconomic aspects.

The need for a noninvasive approach to vitreoretinal disorders is beginning to be met by new methods of altering the state of the corpus vitreus, intended to eliminate untoward effects on the retina and vision. The term "pharmacologic vitreolysis" refers to the use of agents that alter the molecular organization of vitreous in an effort to reduce or eliminate

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Fig. 1. Human corpus vitreus. Photograph of an eye from a 9-month-old child with the sclera, choroid, and retina dissected away, leaving the corpus vitreus attached to the anterior segment. Because of the donor's age, there is a solid gel state to this vitreous, which maintains its shape although the specimen is in room air on a surgical towel. (Specimen courtesy of The New England Eye Bank. Reprinted with permission from Springer-Verlag, New York.[4])

its role in disease. This is achieved by liquefying the gel structure of the corpus vitreus (synchisis) and weakening the adherence of the posterior vitreous cortex to the internal limiting lamina of the retina, leading to separation and collapse of the corpus vitreus away from the retina (syneresis). In similar fashion to endogenous posterior vitreous detachment,[6] the success of pharmacologic vitreolysis depends on inducing these two events simultaneously. Uncoupling these two processes, particularly by inducing liquefaction without weakening vitreo–retinal adherence, may worsen matters significantly. Such a state is present in many conditions that are prone to retinal detachment, such as myopia and various arthro-ophthalmopathies.[7] Synchisis without syneresis may also induce problems at the posterior pole, such as vitreopapillopathies, vitreo–macular traction syndrome, and perhaps even macular holes.

Various agents designed to produce pharmacologic vitreolysis have been tried over the years, but none has met with sufficient success to stimulate widespread use. The table outlines the approaches currently being used. The different agents can be grouped as enzymatic and nonenzymatic. Within the enzymatic group are substrate-specific agents and nonspecific agents. The first of the agents in current use, Plasmin,[8] is a nonspecific protease that can be isolated from the patient's own serum for use at surgery. A phase II clinical trial of this agent is currently being organized in the United States. The other relatively nonspecific, enzymatic agent currently under investigation is Dispase (Gibco, Grand Island, NY).[9] In this issue, this agent is described as having successfully induced posterior vitreous detachment in porcine and human eyes. No evidence of vitreous liquefaction was found, and only postmortem eyes were studied, somewhat limiting the applicability of these findings. A substrate-specific, enzymatic agent that has been in development for a number of years is Chondroitinase. This agent lyses chondroitin sulfate, a molecule that may be important in maintenance of the gel state of the corpus

Table. Pharmacologic Vitreolysis

Type of Vitreolysis	Treatment
Enzymatic	
Nonspecific	Plasmin[3]
	Dispase[9]
Substrate-specific	Chondroitinase[7]
	Hyaluronidase[12,13]
Nonenzymatic[17]	

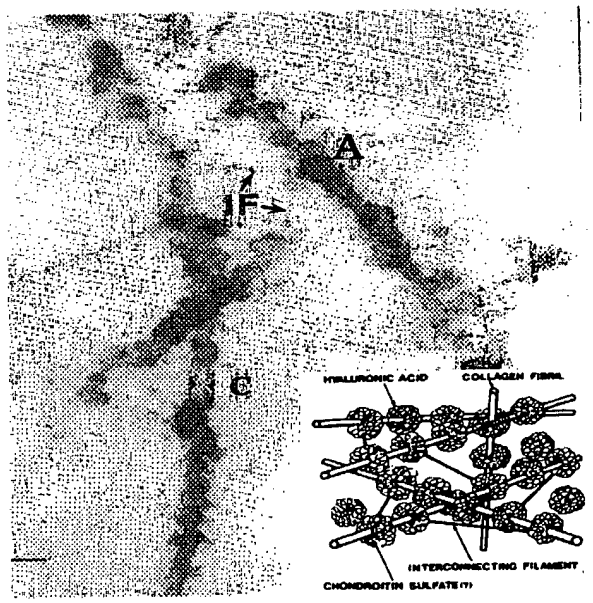

Fig. 2. Ultrastructure of hyaluronan/collagen interaction in bovine vitreous. Bovine vitreous specimen was fixed glutaraldehyde/paraformaldehyde and stained with ruthenium red. Collagen fibrils (C) are coated with amorphous material believed to be hyaluronan. Interconnecting filaments (IF) bridge between collagen fibrils and attach at sites of hyaluronan adhesion to collagen fibrils. The hyaluronan may connect to the collagen fibrils via an intermediary molecule, perhaps chondroitin sulfate. Bar, 0.1 mm. Courtesy of Dr. Asakura. (Reprinted with permission.[10])

vitreus[2,10] and in vitreo–retinal adhesion; hence, there has been considerable interest in the use of this agent. Studies[11] have purported that when used as an adjunct to vitreous surgery, this agent facilitates the removal of premacular membranes. A phase I trial of this agent was completed in the United States nearly 2 years ago; the results await publication in the near future. In this trial, patients with macular holes and others with proliferative diabetic vitreoretinopathy were treated with this agent as an adjunct to vitreous surgery with no significant untoward effects.

As described, pharmacologic vitreolysis can be a useful adjunct to current vitreous surgery techniques. However, pharmacologic vitreolysis can also be performed to replace vitrectomy, as is being attempted with Hyaluronidase for nonclearing vitreous hemorrhage.[12] This application is currently the subject of a phase II clinical trial that has enrolled over 100 subjects in the United States. In this issue, an article[13] suggests that this type of agent may be useful in the induction of posterior vitreous detachment. Should this be true, it may have considerable use in patients with diabetes who are at risk of developing proliferative diabetic vitreoretinopathy, as tremendous benefits can be garnered from inducing molecular vitreolysis before developing this advanced stage of disease.[14] Liquefaction of the corpus vitreus and detachment of the posterior vitreous cortex before the onset of new vessel growth will have a better prognosis than if the vitreous were still attached to the retina.[15,16] Inducing synchisis and syneresis of the corpus vitreus without the use of exogenous enzymes also may be possible, and perhaps safer. In this approach, nonenzymatic agents are used to alter the quatrenary and perhaps even the tertiary conformation of vitreous macromolecules, thereby inducing vitreolysis.[17]

Although the day when vitreous surgery is replaced by noninvasive therapy remains far in the future, these new developments hold great promise. Such approaches should facilitate and enhance present methods of treating vitreo–retinal disorders. Furthermore, these new techniques will likely usher in the time when fewer patients will need the extensive invasive procedures currently being performed. The development of a simpler, cheaper, and less-invasive approach to vitreo–retinal disease eventually may make therapy available to less-privileged people who cannot presently avail themselves of such advanced and costly care.

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Title: Ultrastructure of the vitreoretinal interface following plasmin assisted vitrectomy
Author(s): Gandorfer A; Putz E; Welge-Lussen U; Gruterich M; Ulbig M; Kampik A (REPRINT)
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Journal Subject Category: OPHTHALMOLOGY
Abstract: Aims-To investigate the ultrastructure of the vitreoretinal interface following plasmin induced posterior vitreous detachment.

Methods-Plasmin (1 or 2 U/0.1 ml) was injected into the vitreous cavity of 24 eyes of freshly slaughtered pigs. The 24 fellow eyes received calcium-free and magnesium-free PBS and served as a control. After incubation at 37 degreesC for 30 and 60 minutes, the globes were placed in fixative and hemisected. Specimens for light, scanning, and transmission electron microscopy were obtained from the posterior pole, the equator, and the vitreous base using a corneal trephine.

Results-All plasmin treated eyes showed posterior vitreous detachment. However, the inner limiting membrane (ILM) was covered by remnants of cortical vitreous at the posterior pole and at the equator. There was a direct correlation between the concentration and exposure times of plasmin and the degree of vitreoretinal separation. Eyes exposed to 1 U plasmin for 30 minutes had a dense network of residual collagen fibrils while those exposed to 1 U plasmin for 60 minutes had only sparse collagen fibrils covering the ILM. Eyes treated with 2 U plasmin for 60 minutes had a smooth retinal surface, consistent with a bare ILM. At the vitreous base there was no vitreoretinal separation. In all control eyes the vitreous cortex was completely attached to the retina. There was no evidence of retinal damage in any plasmin treated eye.

Conclusion-Plasmin induces a cleavage between the vitreous cortex and the ILM without morphological changes to the retina. In contrast with previous reports, plasmin produces a smooth retinal surface and additional surgery is not required in this experimental setting. The degree of vitreoretinal separation depends on the concentration and length of exposure to plasmin.

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Seminars in Ophthalmology
Vol-15
Issue-1
" Tissue Plasminogens Activator in the Treatment of Vitreoretinal Diseases"
Kamei, M.; Estafanous, M.; Lewis, H.
Pages:44-50
Year-2000

Retina
Vol-19
Issue-1
" Posterior Vitreous Detachment Induced by Injection of Plasmin and Sulfur Hexafluoride in the Rabbit Vitreous"
Hikichi, T.; et al
Pages:55-58
Year-1999

For **Matthew F. DeSanto, AU 3763, 305-3292, US serial NOT GIVEN**.

Thanks,

Terry Solomon
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CP2, 2C08

Tissue Plasminogen Activator in the Treatment of Vitreoretinal Diseases

Motohiro Kamei, Marc Estafanous, and Hilel Lewis

Tissue plasminogen activator (tPA) is a thrombolytic agent that activates plasminogen into plasmin almost exclusively in the presence of fibrin. Intraocular injection of tPA has been proposed for the treatment of vitreoretinal diseases, such as vitreous hemorrhage, postvitrectomy fibrin formation, submacular hemorrhage, retinal vascular occlusive disorders, suprachoroidal hemorrhage and endophthalmitis. Currently, intraocular tPA is only used in the treatment of postvitrectomy fibrin formation and submacular hemorrhage. For other indications, tPA has not been shown to be safe or effective. This article reviews the use of tPA in the treatment of vitreoretinal disorders.

TISSUE PLASMINOGEN activator (tPA) is a thrombolytic agent that activates plasminogen into plasmin almost exclusively in the presence of fibrin. The first clinical application of tPA was in the setting of acute myocardial infarction.[1,2] Since that time, tPA has been used to treat pulmonary embolism,[3] unstable angina pectoris,[4] deep vein thrombosis,[5] peripheral arterial occlusion,[6] and stroke[7] as well as various ocular disorders.

The safety and efficacy of intraocular injection of tPA has been well established. Animal models of several ocular disorders have been investigated including intraocular fibrin deposition,[8] postvitrectomy fibrin formation,[9] hyphema,[10] fibrin formation in glaucoma filter blebs,[11] vitreous hemorrhage[12-14] subretinal hemorrhage,[15-20] suprachoroidal hemorrhage,[21,22] and retinal arterial thrombi.[23] Clinically, intraocular tPA has been safely used for the treatment of fibrin formation after glaucoma filtering procedure,[24,25] postcataract surgery anterior chamber fibrin formation,[26,28] hyphema,[29,30] postvitrectomy fibrin pupillary block glaucoma,[31] postvitrectomy fibrin formation,[32-36] postvitrectomy vitreous hemorrhage,[37] submacular hemorrhage,[38-49] central retinal vein occlusions,[50,51] and other disorders.[52-55] This review concentrates specifically on the use of tPA in vitreoretinal disorders.

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BIOCHEMISTRY AND PHYSIOLOGY

tPA is a relatively large molecule with a molecular weight of about 70 kD, composed of 527 amino acids[56] (Fig 1). It is a serine protease, which cleaves the Arg560-Val561 peptide bond of plasminogen. The NH_2-terminal region of tPA contains several domains which are homologous to the finger domains of fibronectin, epidermal growth factor and kringle domains of plasminogen and are thought to be involved in its fibrin specific binding property.[57-60] The COOH-terminal region is homologous to that of other serine proteinases and contains the catalytic site.[61] There are 2 forms of tPA, a single chain form and a two chain form. tPA is secreted in its single form and then rapidly converted by plasmin to its two chain form by hydrolysis of the Arg275-Ile276 peptide bond. Both forms of tPA show similar pharmacokinetic properties when used at equivalent doses.[1] Commercial tPA (Activase, Genentech; Actilyse, Boehringer) is a single chain form produced by recombinant DNA technology using Chinese hamster ovary (CHO) cells and is supplemented with a large amount of L-arginine to prevent self-degradation.

The half-life of intravenously infused tPA is as short as a few minutes. It is cleared from the circulation mainly by the liver.[1] Jaffe et al[62] determined the half-life of intravitreally injected tPA to be about 4 to 6 hours in rabbit eyes without an intravitreal fibrin clot, and about 10 to 12 hours in the presence of fibrin clots.

Among the plasminogen activators, tPA in particular has a highly specific affinity for fibrin,[1,63,64] and enhances the binding of plasminogen to a fibrin clot.[65] The activation of plasminogen into plasmin by tPA is potentiated over 600-fold in the presence of fibrin.[63] Furthermore, while free plasmin is rapidly neutralized by alpha2-antiplasmin, inactivation of plasmin on a fibrin surface is slow.[66] The combination of these molecular interactions allows

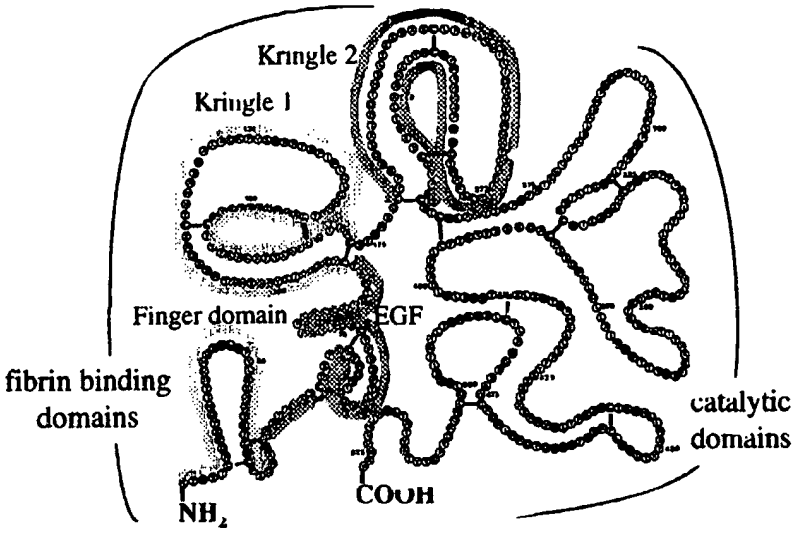

Fig 1. The primary structure of human tissue plasminogen activator. (Modified and reprinted with permission.[1])

fibrinolysis by tPA to occur almost exclusively in fibrin clots. Clinically, its fibrin specificity leads to less depletion of plasma fibrinogen, and fewer degradation products in plasma compared to streptokinase, urokinase or nasaruplase, when given intravenously.[64]

CLINICAL APPLICATIONS

Postvitrectomy Fibrin Formation

Of 106 eyes with postvitrectomy severe fibrin formation treated with tPA, complete fibrin resolution was achieved in 86 eyes (81%).[32-37] The eyes were treated by injection of 3 to 30 μg of tPA into either the anterior chamber or the vitreous cavity. The time required to complete fibrin resolution was less than 4 hours in all studies.

Vitreous Hemorrhage

Several studies indicate that intravitreal injection of tPA is effective in the clearance of experimental vitreous hemorrhage.[12-14] Koutsandrea et al[37] demonstrate that 3 cases of vitreous hemorrhage after vitrectomy received intravitreal tPA injection but the hemorrhage persisted unchanged. Further randomized clinical trials with large number cases are necessary.

Submacular Hemorrhage

Submacular hemorrhage has a poor visual prognosis, especially when the hemorrhage is thick or associated with age-related macular degeneration (AMD).[67] The mean final visual acuity in a clinical study reported by Bennett et al[67] was 20/1700. In another clinical study describing the natural history of submacular hemorrhage associated with AMD, retinal arterial macroaneurysms and ocular histoplasmosis syndrome, visual recovery rates were less than 50%.[68] Experimental studies have shown fibrin mediated irreversible damage to the outer retina within 24 hours of onset.[15,69]

Surgical removal of subretinal hemorrhage by forceps was first reported by Hanscom and Diddie[70] in 1987. Mechanical removal of the clot was associated with a poor visual result probably because fibrin interdigitates between the hemorrhagic clot and photoreceptors, and the removal resulted in significant damage to the outer retina.

Subretinal Injection

Experimental studies showed that subretinal injection of tPA was safe and effective in promoting the clearance of experimentally produced subretinal hemorrhage.[15] tPA also decreased the damage to the overlying retina caused by the subretinal blood. A dose of 25 μg/0.1 mL was effective in accelerating the clearance of subretinal hemorrhage and doses up to 50 μg/0.1 mL showed no morphological evidence of toxicity or inflammation. The beneficial effect can probably be explained by lysis of interphotoreceptor fibrin; reduced adherence of the clot to the outer retina might facilitate clearance of the hemorrhage and minimize the shearing damage to the outer segments of the photoreceptors.

Table 1. Results of tPA Assisted Surgical Removal of Submacular Hemorrhage Associated With AMD

	No. of Eyes	Initial VA (mean)	Final VA (mean)	Improvement in VA (%)
Lewis	24	HM-CF	20/180	83
Ibanez et al	19	20/530	20/480	47
Lim et al	16	9/200	20/286 (best VA = 20/129)	56
Kamei et al	22	5/200	20/164	82
Moriarty et al	14	HM	5.5/45	86
Kamei et al	92	20/1200	20/175	78

Abbreviations: VA, visual acuity; HM, hand motion; CF, counting fingers.

Clinically, subretinal injections of tPA allow submacular hemorrhages to be liquefied and evacuated through a small retinotomy, which reduces surgery-induced damage to the neural retina and retinal pigment epithelium.[38-44] The results of tPA assisted surgical removal of submacular hemorrhage associated with AMD in recent reports are summarized in Table 1. Our results in 92 patients who underwent tPA assisted removal of submacular hemorrhage showed that final visual acuity was 20/200 or better in 56% of the eyes and the visual acuity improved in 78%.[45] Submacular hemorrhage associated with retinal arterial macroaneurysms have also been successfully treated by surgical removal with subretinal tPA.[46]

Postoperative complications with this technique include disciform scar formation due to persistent choroidal neovascularization (11%), recurrence of the hemorrhage (5% to 18%), retinal detachment and PVR (4% to 14%), epiretinal membrane formation (14%), and cataract formation (12% to 13%).

Prognostic factors include duration of the hemorrhage, area of the hemorrhage, and where a hemorrhagic detachment of the retinal pigment epithelium is present.[45] Duration of submacular hemorrhage of 7 days or less showed a statistically significant association with postoperative visual acuity of 20/200 or better ($P = .02$, $n = 92$). Subretinal hemorrhage localized within the temporal arcades was also associated with 20/200 or better postoperative vision ($P = .02$, $n = 92$). The absence of hemorrhagic retinal pigment epithelial detachment was associated with 5/200 or better postoperative vision ($P = .04$, $n = 92$).

Although we do not yet have precise information on the natural history of submacular hemorrhage, we currently consider tPA assisted surgical removal of submacular hemorrhage in the following cases: A submacular hemorrhage that is (1) less than 8 days old, (2) 500 μm thick or more and (3) localized within the temporal arcades, (4) in a patient who had good visual acuity immediately before the submacular hemorrhage developed. Contraindications for surgical evacuation include (1) duration of hemorrhage greater than 3 weeks, (2) thin and small hemorrhages, and (3) poor visual acuity before the bleeding. Other factors, such as conditions of the fellow eye, etiology and duration, should be considered when considering surgery.

Intravitreal Injection

Although surgical evacuation of submacular hemorrhage with tPA has been proven to be effective and safe in the clinical trials, vitreoretinal surgery, with its possible risks and complications, is required to introduce tPA into the subretinal space. In addition, intraoperative waiting time for fibrinolysis to occur is at least 45 minutes,[38-44] which can make the surgery inconvenient to both patient and surgeon.

Intravitreal injection of tPA has therefore been tried as an alternative, more practical approach.[19,20,47,48] The efficacy of this approach, however, has not been tested in clinical trials with appropriate controls. Retina damaged by subretinal hemorrhage might allow tPA to penetrate into the subretinal space, and intravitreally injected tPA did change the color of the clotted blood in an animal model;[19,20] however, the reports did not specifically show that the clots had been lysed, nor did they present any evidence that tPA did in fact penetrate the neural retina into the subretinal space and lyse fibrin beneath the retina. Because tPA has a rather large molecular weight of approximately 70 kd, it generally cannot easily diffuse across biological membranes.[71] In fact, tPA is theoretically too large to allow it to cross the neural retina into the sub-

retinal space when considering that the pore size of the outer limiting membrane is between 30 and 36 angstrom, which correspond to molecules of around 50 to 60 kd.[72]

To study this issue further, we conducted a study to determine whether tPA injected into the vitreous could penetrate the neural retina and enter the subretinal space.[73] We injected a mixture of fluorescein isothiocyanate (FITC)-labeled tPA (70 kd) and a small molecule control, rhodamine-B isothiocyanate (RITC)-labeled dextran (20 kd), into the vitreous cavity of rabbit eyes. The distribution of the labeled molecules was examined with epifluorescent microscopy. We also evaluated tPA pharmacokinetics in eyes with a subretinal clot induced by injecting autologous blood into the subretinal space through the sclera. Our results show that tPA was present at the vitreal surface of the retina in a linear array and was observed neither in the neural retina nor in the subretinal clot. The dextran control, on the other hand, had diffused throughout the neural retina (Fig 2). Although intravitreal tPA cannot penetrate the intact neural retina, the FITC signal of tPA in the neural retina was observed in a couple of eyes with intraretinal hemorrhage. Each of these eyes had vitreous hemorrhage, and microscopic retinal tears may have been present. Therefore, we conclude that intravitreal tPA does not diffuse through the neural retina to reach a subretinal clot and that there is no scientific rational for the treatment of submacular hemorrhage with intravitreal tPA. Our results support a report which showed shifting of subretinal hemorrhage after intravitreal injection of air without tPA.[74]

Surgical Excision of Choroidal Neovascular Membranes

Initial reports indicated a possible benefit from the use of tPA prior to surgical removal of choroidal neovascular membranes (CNVMs).[49,52] One member of our group (HL), however, showed in a large randomized, prospective, double-blind study of 80 patients with AMD, that there is no benefit in the use of subretinal tPA before excision of subfoveal CNVMs.[75]

Retinal Vascular Occlusion

Retinal vein occlusions are second only to diabetes as causes of retinal vascular disease in the United States.[76] The cause of retinal vein occlusions has been shown to be thrombus formation within the retinal vein.[77,78] Intravenous thrombolytics, eg, streptokinase and tPA, have been used in an attempt to treat central retinal vein occlusion. The potential risk of serious complications, including a high rate of vitreous hemorrhage with streptokinase[79] and a risk of death from stroke with tPA,[80,81] have limited their use as a routine therapy for retinal vein occlusions. One investigator has reported the direct injection of tPA into a retinal vein resulting in a subjective improvement in vision. However, the technique is technically challenging and requires specialized equipment.[50]

The use of intravitreal tPA for the treatment of retinal vein occlusions is promising. One issue that remains to be addressed regarding the use intravitreal injection of tPA in this setting is the ability of tPA to cross the blood-retinal barrier (BRB) to reach the thrombus. It has been shown that the BRB is compromised after retinal vein occlusion,[82] and our group has recently shown that tPA can penetrate the neural retina in the presence of intraretinal hemorrhage.[73] We are currently investigating the ability of tPA to penetrate the BRB in the setting of retinal vein occlusions (work in progress). Lahey et al[51] have used intravitreal tPA in patients with central retinal vein occlusion and reported a rate of 70% improvement or stabilization in vision. The study was not controlled.

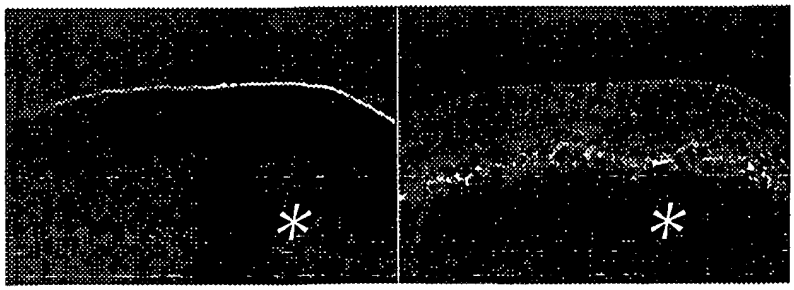

Fig 2. Epifluorescent micrographs of a rabbit eye in which a subretinal clot (asterisks) was experimentally induced. The FITC signal (= tPA) is present in a linear array along the surface of the retina and is absent from the neural retina and the subretinal clot (left), whereas the RITC signal (= 20 kd dextran) diffuses through the retina in the same frame (right).

The use of tPA in the setting of retinal artery occlusion has not been well explored. Experimentally, intravenous tPA was used successfully to lyse laser-induced retinal arterial thrombi in a rat model.[23]

Proliferative Diabetic Retinopathy

Hesse and Kroll[54,55] have shown in 2 recent studies that intravitreal injection of tPA can successfully induce posterior vitreous detachment (PVD) in eyes with proliferative diabetic retinopathy. Their initial study was a blind randomized trial of intravitreal tPA or placebo injection as an adjunct to vitrectomy. The operating surgeons were able to identify eyes which tPA had been injected in all cases.[54] They then reported successful induction of PVD in 10 of 11 eyes (91%) after injection of intravitreal tPA compared with 1 of 9 eyes (11%) in the control group. Injections were given 8 weeks before the scheduled vitrectomy.[55]

Other Vitreoretinal Diseases

Intraocular injection of tPA in experimental Staphylococcal endophthalmitis has been shown to be of no benefit in preventing fibrin formation.[83-85]

MacCumber et al[53] successfully used anterior chamber injections of tPA to prevent peripheral iridectomy occlusion in 12 eyes with silicone oil after retinal detachment surgery.

Experimental suprachoroidal hemorrhage has been successfully treated by tPA, including intravenously, in rabbits.[21,22] Subconjunctival injection of tPA, however, was not successful in the same model.[86]

CONCLUSION

Because of its fibrin-specific fibrinolytic activity, tPA is widely used in the treatment of various kinds of vitreoretinal diseases. Usage of tPA can be refined and applied to more ocular disorders such as retinal vein occlusion and posterior vitreous detachment in near future. Although the optimum dose has not been determined yet, low-dose application (3 to 10 μg) is reported to be effective in animal models and clinical trials.[14,29,30,34]

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Seminars in Ophthalmology
Vol-15
Issue-2
" Enzymatic Vitreous Surgery"
Trese, M.T.
Pages:116-121
Year-2000

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Enzymatic Vitreous Surgery

Michael T. Trese

Enzymatic manipulation of the vitreous and vitreoretinal juncture is currently in the process of being evaluated in many centers around the world. The goals of such manipulation are either to disinsert the posterior hyaloid from the retina surface in an atraumatic, very clean, cleavage plane or, at this point, to try to disinsert the peripheral vitreous from the neurosensory retina. In addition, enzymatic manipulation of the central vitreous in terms of liquefaction has also been evaluated. Although this is certainly the beginning of this type of vitreal surgery, adjuvant or alternative, it does appear to be a new and exciting area of vitreoretinal surgery.

ALTHOUGH THERE ARE probably many reasons to pursue enzymatic vitreous surgery, the common goal for enzymatic vitreous surgery is the manipulation of collagen tissue in the vitreous cavity with minimal negative effects to the retinal surface and the eye as a whole. The concept of using enzymes as an adjunct to standard vitreous surgery or as an alternative to standard vitreous surgery is in the process of being explored. The potential benefits of enzymes would be less destruction to the optic nerve, less destruction to the anterior retinal surface, a very clean separation between internal limiting lamina and the posterior hyaloid, which often is the circumstance envisioned in the surgeon's mind, yet, frequently, islands of collagenous tissue or inadvertent removal of the internal limiting lamina is most likely achieved.

POTENTIAL ENZYMATIC CANDIDATES

Many enzymes have been used in ocular surgery over the years. α-chymotrypsin was used for zonular digestion in the past and was used for many years as an adjunct in cataract surgery. Wydase has been used with retrobulbar injection to help spread the anesthetic agent throughout the orbit. It is used even today for that purpose. Enzymes that have been suggested for adjunctive therapy to vitreous surgery have been chondroitinase, hyaluronidase, dipase, and plasmin enzyme. The issues of enzymatic assembly must be considered including the construction of recombinant versus autologous enzymatic agents. In addition, agents that activate endogenous enzymes have been considered such as tissue plasminogen activator (t-PA). All of these biochemical pathways have as their goal the potential to manipulate either central vitreous collagen or the vitreoretinal interface. The enzymes that seem most promising for manipulation of the vitreoretinal interface seem to be plasmin enzyme or chondroitinase. The enzyme that has been focused on primarily to deal with the manipulation of the central vitreous cavity has been hyaluronidase. The merits of this type of therapy certainly have foundation in patient benefit as a final common goal. However, considerations of added cost must also be considered. The more costly recombinant forms of enzymatic therapy may not be as advantageous as an autologous preparation because it most likely can be produced at a much lower cost. The idea of manipulation of endogenous substances such as with the use of t-PA has also been proposed and been shown in animal models to be effective. Seemingly, the mechanism of t-P is the activation of plasminogen to plasmin enzyme with the final common pathway being the plasmin enzyme manipulating at least the vitreoretinal juncture. Animal studies have supported the role of chondroitinase as well as plasmin enzyme on the manipulation of the vitreoretinal juncture. The role of the t-PA activating plasmin enzyme has also been supported by animal studies. Although the majority of animal work has been performed in rabbit models, the pig has also been studied using plasmin and nonhuman primates have been studied using chondroitinase.

BACKGROUND OF ENZYMATIC MANIPULATION OF THE VITREOUS

Both experimental studies and clinical observations confirm the central role of the vitreous in the pathobiology of ocular trauma. This suggests a potentially beneficial effect of vitrectomy in trauma.[1,2] Although the optimal timing of vitrectomy remains controversial, separation of the vit-

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reous from the retina is a cardinal goal of vitrectomy surgery in trauma. Despite continuing improvements in vitrectomy instrumentation and techniques, complete removal of the cortical vitreous from the internal limiting membrane of the retina is often difficult, particularly in young patients who constitute the majority of ocular trauma victims. In this population, the vitreous is often strongly adherent, and attempted surgical removal of the cortical vitreous from the retina may cause further retinal damage. Indeed, 1 of the major arguments for delayed vitrectomy after trauma is to allow for the development of a spontaneous posterior vitreous detachment (PVD).[1] This usually occurs within 1 to 2 weeks after penetrating trauma and is thought to result from trauma-related breakdown of the blood-ocular barrier with attendant migration of macrophages and plasma components into the vitreous. These macrophages release enzymes that break down the vitreous gel and lyse the vitreoretinal bond in conjunction with mechanical traction forces. The rationale underlying pharmacological manipulation of the vitreous is to identify agents that can cleave the vitreoretinal interface without damaging the retina. This may be accomplished with an agent that can dissolve the molecular glue responsible for the adhesion of the vitreoretinal bond. The ideal agent would also be capable of liquefying the formed vitreous gel. Taking a cue from nature, enzymes are the most likely candidates for such an agent and in fact have been considered for nearly 50 years. The next section reviews the enzymes that have been considered in the pharmacological manipulation of the vitreous.

SPECIFIC AGENTS FOR MANIPULATION OF THE VITREOUS

Plasmin

The use of plasmin for induction of vitreoretinal separation derives from the observation in diabetes that vitreoretinal separation usually begins near neovascularization. Neovascularization is characterized by a high degree of proteolysis, which is necessary for the growth and development of new vessels. An important enzyme in proteolysis is the plasminogen activator urokinase, which generates plasmin. Plasmin is a nonspecific protease that de-

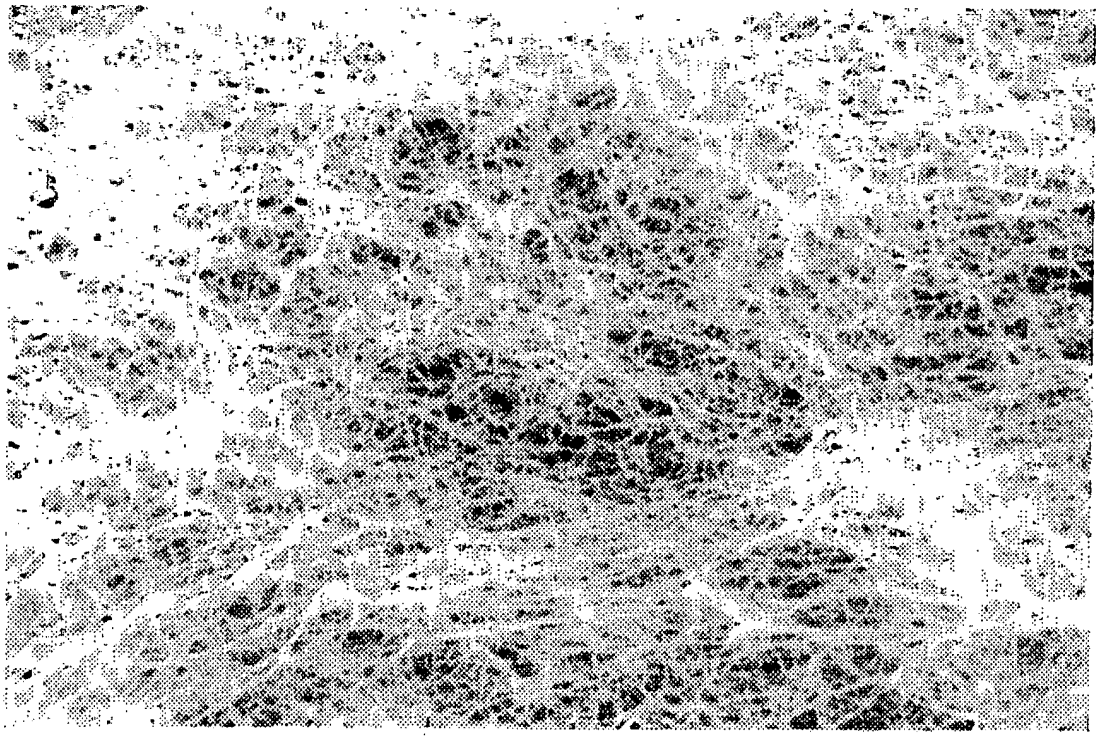

Fig 1. Scanning electron micrograph of a control eye of a rabbit viewing the anterior retinal surface.

grades a variety of substances in the extracellular matrix, including laminin and fibronectin, which have been implicated in the vitreoretinal adhesion. This observation led to the experimental evaluation of plasmin in an animal model and, more recently, to human trials.

In the rabbit model, plasmin facilitates the separation of the vitreous from the retina.[3] A dose of 1 U of plasmin injected intravitreally followed up by vitrectomy 60 minutes later enhances separation of the cortical vitreous from the internal limiting lamina (ILM) as shown by scanning electron microscopy (Figs 1, 2, and 3). Electroretinography at 7 days is comparable between control and plasmin-treated eyes. There also is no evidence of toxicity on light microscopy.

Plasmin is now being used on an investigational basis in humans.[4] To date, most of the patients treated have been infants, children, or young adults. It is the subjective impression of the operating surgeons that plasmin facilitates separation of the vitreous from the retina. No hemorrhagic or toxic complications have been seen. However, randomized trials have not yet been performed. The potential applicability of plasmin treatment to ocular trauma is readily apparent. Plasmin could be used after primary repair of penetrating trauma to accelerate formation of PVD. Alternatively, plasmin could be used intraoperatively to facilitate vitreoretinal separation and minimize potential complications from iatrogenic vitreoretinal separation.

Complete removal of the posterior hyaloid from the retinal surface is particularly difficult in young eyes. Iatrogenic separation of the vitreous during macular hole surgery has been implicated in the formation of postoperative visual field defects.[5,6] Lysis of the vitreoretinal adhesion with plasmin may minimize the development of postoperative visual field defects.[5]

Our group has used plasmin in the repair of pediatric traumatic macular holes with excellent results. In a small series of 10 eyes with traumatic macular holes, plasmin appeared to facilitate vitreoretinal separation without retinal complications. All 10 eyes had closure of the macular hole and visual acuities ranging from 20/20 to 20/50.

Intraoperatively, plasmin appears to induce liquefaction of the formed vitreous gel. This suggests

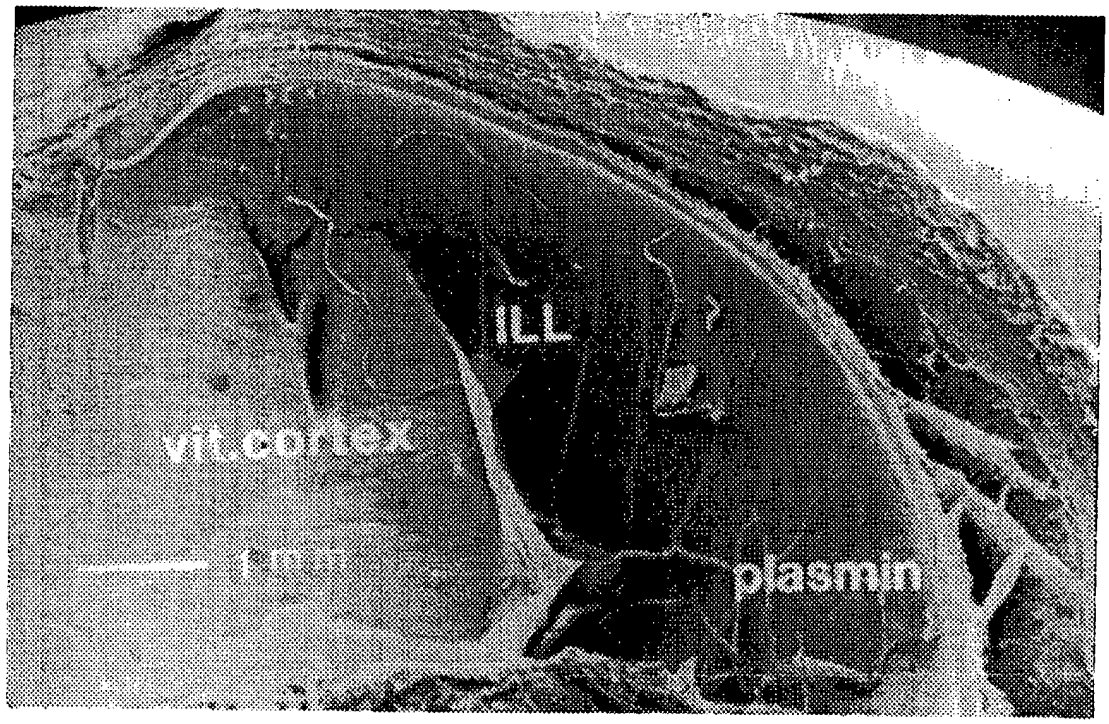

Fig 2. Scanning electron micrograph of rabbit eye injected with plasmin enzyme alone. The vitreous cortex is separated from the retinal surface.

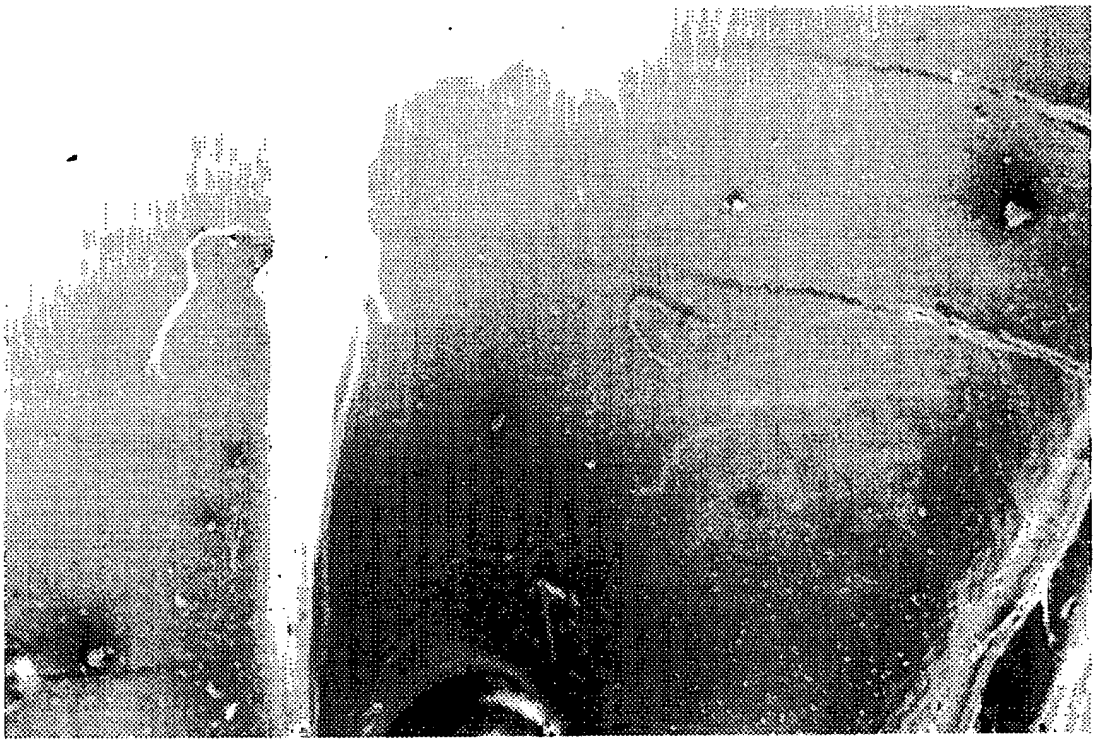

Fig 3. Scanning electron micrograph of a rabbit eye with plasmin enzyme injected and the eye irrigated by vitreous surgery techniques.

the possibility of using plasmin in conjunction with fluid-gas exchange to remove vitreous without vitrectomy. Animal studies suggest this scenario is feasible, but further work is required.[7]

Currently, we are using autologous plasmin because of the risks inherent to pooled human plasma products. The plasmin is isolated from the patient's blood as follows. Thirty to 40 mL of anticoagulated blood is centrifuged to isolate the plasma. The plasma is then run on an activated lysine column, which binds the plasminogen. The plasminogen is then dilated off the column and converted to plasmin by streptokinase. This process consistently yields approximately 1 U of plasmin activity. The current process requires 48 hours to isolate the plasmin and assure sterility. The plasmin is injected through the pars plana into the vitreous cavity 15 minutes before vitrectomy. Usually 0.4 U in 0.1 to 0.2 mL is injected. Further work is ongoing to optimize the dose and exposure time of plasmin therapy. A randomized clinical trial of autologous plasmin therapy in vitrectomy is being prepared.

Tissue Plasminogen Activator

The endogenous formation of plasmin is regulated by an intricate balance between plasminogen activators and plasminogen activator inhibitors. Pharmacological stimulation of plasmin can be achieved with plasminogen activators, which convert plasminogen into plasmin. t-PA has been used intraocularly since 1987 for the treatment of postsurgical fibrin formation[8] and more recently for the treatment of subretinal hemorrhage.[9-11] Although animal studies show some toxicity at doses of 50 μg and above,[12] clinical experience suggests t-PA is nontoxic in the human eye at doses of up to at least 50 μg and perhaps as high as 100 μg. The half-life of t-PA after injection of 25 μg in the vitrectomized phakic rabbit eye is approximately 12 hours.[13] The limited human pharmacokinetic data are consistent with this data. However, the effective half-life of t-PA probably exceeds the half-life of the free t-PA because of fibrin binding and the prolonged effect of plasmin.

t-PA is available as a recombinant DNA product for human use. Because plasmin is not readily available, t-PA has been used as a biochemical adjunct in vitrectomy surgery for proliferative diabetic retinopathy.[14] A dose of 25 μg in 0.1 mL is injected into the vitreous 15 minutes before vitrectomy. The t-PA converts endogenous intraocular plasminogen into plasmin. The plasminogen is present in the eye because of the breakdown of the blood ocular barrier by both the diabetic state and prior cryopexy of the vitreous base. In a small series, t-PA therapy appeared to facilitate separation of the vitreous cortex from the retina in a manner similar to plasmin. There were no intraoperative or postoperative hemorrhagic complications. Additional investigations with t-PA adjunctive therapy are ongoing. If these preliminary results are confirmed t-PA also may be useful in trauma because trauma usually results in breakdown of the blood ocular barrier.

Chondroitinase

A 240-kd chondroitin sulfate proteoglycan is associated with the vitreoretinal interface.[15] The greatest immunoreactivity of this proteoglycan is at the vitreous base and the optic nerve, suggesting a role in vitreoretinal adhesion. Chondroitinase lyses this proteoglycan and has been studied as an adjunct in vitrectomy. In cynomolgus monkeys and human organ donors, intravitreal injections of chondroitinase separated the vitreous from the retina without damage to the ILM.[16,17] Chondroitinase has been studied in phase I human trials but no results have been yet reported. Chondroitinase appears to be a promising agent for pharmacological manipulation of the vitreous.

The use of plasmin, t-PA, and chondroitinase in vitrectomy surgery has shown the feasibility of pharmacologically manipulating the molecular biology of the vitreous and opened the door to an exciting new frontier in vitreoretinal surgery. Although this preliminary experience is encouraging, additional work is required before the promise of enzyme-assisted vitrectomy becomes a clinical reality.

HUMAN EXPERIENCE WITH ENZYMATIC VITREOUS MANIPULATION

Although human experience in the literature is limited, 2 series of manipulation of the vitreoretinal juncture using plasmin enzyme have been reported. The first deals with traumatic macular holes that show that autologous plasmin enzyme does appear to be effective in this very small series of patients. The enzyme itself is seemingly helpful in this population of younger patients who have experienced traumatic macular holes and have, indeed, a very strong vitreoretinal juncture. The enzyme allows a less-traumatic manipulation of the vitreoretinal interface. The second series of surgically manipulated stage 3 macular holes suggests that macular hole surgery may be performed in an easier, safer, and quicker time frame. Both were very small uncontrolled studies and require a larger study to confirm the findings.

The hope of this study is that macular hole surgery may at some time be able to be made safe and quick enough that it may be able to be performed in an office setting using enzymatic manipulation of the vitreoretinal interface as well as small gauge instruments allowing manipulation of the vitreous cavity having been treated with plasmin or another enzyme or a combination of enzymes that may both liquefy the vitreous gel and manipulate the vitreoretinal interface. This particular type of technique allows the vitreous to be able to be removed through a smaller gauge instrument not requiring surgical suturing or creation of a larger wound in the sclera and conjunctiva. This type of human experience is only the beginning of enzymatic manipulation of vitreous collagen. Collagen, after all, does lend itself to enzymatic control more than cellular structures. The goal must remain to achieve results at least as good as conventional surgical techniques, but accompanied by less trauma to neighboring cellular structures. This type of biochemical manipulation of ocular structures I believe represents only the beginning of further cell surface manipulation by biochemical agents. This use of enzymatic materials may allow the vitreoretinal surgeon of the 21st century to be able to perform finer, more meticulous types of manipulation within the vitreous cavity, both of the vitreoretinal interface, vitreovascular interfaces, and, perhaps, central vitreous structure as well.

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Jacob, R becca (ASRC) 422768

From: STIC-ILL
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-----Original Message-----
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Ophthalmologe
Vol-98
Issue-12
" Using Enzymes in the Posterior Eye Segments. Current Status and Future Possibilities"
Hesse, L.
Pages:1176-1180
Year-2001

American Journal of Ophthalmology
Vol-133
Issue-1
"Vitreoretinal Morpholgy of Plasmin-treated Human Eyes"
Gandorfer, A.; et al
Pages:156-159
Year-2002

For **Matthew F. DeSanto, AU 3763, 305-3292, US serial NOT GIVEN.**

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Ophthalmologe
2001 · 98:1176–1180 © Springer-Verlag 2001

L. Hesse · Augenklinik der Philipps-Universität Marburg

Einsatz von Enzymen im hinteren Augenabschnitt

Derzeitiger Stand und zukünftige Möglichkeiten

Zusammenfassung

Die enzymatische Behandlungsmethode von Erkrankungen im hinteren Augenabschnitt findet zunehmende Verbreitung. Derzeit wird durch intravitreal injizierten Gewebeplasminogenaktivator eine subretinale Blutung lysiert und mittels eines intravitreal injizierten Gases mechanisch aus der Makula verdrängt. Jüngste morphometrische Analysen konnten eindeutig eine subretinale Fibrinolyse nach intravitrealer Injektion von Gewebeplasminogenaktivator nachweisen. Damit konnte erstmals eine aufwendige Pars-plana-Vitrektomie durch ein enzymatisches Therapieverfahren ersetzt werden. Klinische Erfahrungen mit der Pars-plana-Vitrektomie zeigten, dass eine vollständige Entfernung des Glaskörpers einen günstigen Einfluss auf den Verlauf von vasoproliferativen vitreoretinalen Erkrankungen hat. Therapeutische Ziele einer enzymatischen Behandlung des Glaskörpers zielten daher entweder auf die Verflüssigung des Glaskörpers oder die Trennung der hinteren Glaskörperrinde von der Netzhaut. Insbesondere durch jüngste Erkenntnisse zur Pathophysiologie vasoproliferativer vitreoretinaler Erkrankungen ergeben sich neue therapeutische Optionen. Künftig wäre eine Behandlung des diabetischen Glaskörpers mit geeigneten Enzymen denkbar, um die Entstehung eines proliferativen Verlaufs der diabetischen Retinopathie zu verhindern.

Schlüsselwörter

Glaskörper · Enzyme · Protease · Subretinale Blutung · Proliferative diabetische Vitreoretinopathie · Therapie

Wir verfügen derzeit über zwei unterschiedliche Prinzipien zur Behandlung von Erkrankungen des hinteren Augenabschnitts. Neben den differenzierten chirurgischen Verfahren (Abb. 1) und der Pharmakologie wird gegenwärtig die Gentherapie als dritte Komponente entwickelt. Die beiden vergangenen Jahrzehnten waren geprägt durch neue technische Entwicklung der Pars-plana-Vitrektomie und wachsende klinische Erfahrungen in der chirurgischen Behandlung vitreoretinaler Erkrankungen. Höhepunkt ist die Makularotation mit kombinierter Muskelchirurgie als derzeit chirurgisch aufwendigster Eingriff. Weniger spektakulär ist dagegen ein Wechsel von den invasiven zu den weniger invasiven Eingriffen. Gegenstand der Forschung sind gegenwärtig der Einsatz von Enzymen bei der Behandlung der subretinalen Blutung und der proliferativen diabetischen Vitreoretinopathie.

Enzymatische Behandlung einer subretinalen Blutung

Die akute subretinale Blutung wird heute nicht mehr mit aufwendiger Glaskörperchirurgie behandelt, sondern durch die intravitreale Injektion eines Gewebeplasminogenaktivators (engl. „tissue plasminogen activator", TPA) und eines expandierenden Gases [5, 8, 13, 19]. Das injizierte TPA verflüssigt zunächst das Koagel, und anschließend wird das Blut durch die Gasblase bei gleichzeitiger Kopfneigung aus der Makula verdrängt (Abb. 2). Obwohl die Therapie wirksam ist, sind einige Punkte strittig.

Strittige Fragen

Kann das TPA-Protein aufgrund seiner Größe (70 kDa) überhaupt die Netzhaut passieren, um eine subretinale Fibrinolyse zu induzieren?

Anhand morphometrischer Analysen gelang kürzlich der klinische Nachweis der subretinalen Fibrinolyse nach intravitrealer Injektion von TPA. Ausgewertet wurden die Ausdehnung und der geometrische Mittelpunkt (Abb. 3) von subretinalen Blutungen (n=13) präoperativ, nach TPA- und nach Gasinjektion. Die Behandlung bestand in der intravitrealen Injektion von 50 µg/50 µl TPA (Actilyse, Thomae, Biberach a.d. Riß), gefolgt von einer Gasinjektion (0,2–0,3 ml SF6) einen Tag später [14]. Als klinisches Zeichen der subretinalen Verflüssigung des Koagels wurde die Vergrößerung der Fläche der Blutung gewertet, die sich einen Tag später signifikant ($p<0{,}001$) um durchschnittlich 36,9% vergrößert hatte. Der fibrinolytische Effekt korrelierte mit der präoperativ durch Ultraschall bestimmten Dicke der subretinalen Blutung. Bei flachen Blutungen (<0,8 mm) war keine nennenswerte Änderung nach TPA-Injektion zu beobachten, während dicke Blutungen eine deutliche Verflüssigung zeigten. Offensichtlich entstehen durch die hämorrhagische Abhebung der Netzhaut während der akuten Blutung

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L. Hesse

The present and future use of enzymes in the posterior segment

Abstract

The first investigations to treat diseases of the posterior segment enzymatically started 40 years ago. To treat acute subretinal hemorrhage a pneumatic displacement through intravitreally injected gas after enzymatically induced subretinal fibrinolysis (TPA) is recommended. Recent morphometric analysis clearly demonstrated a subretinal fibrinolytic effect after intravitreal injection of TPA. Obviously TPA crosses the retina through microlesions that develop through elevation of the retina during acute bleeding. For the first time pars plana vitrectomy was superseded by a simple and gentle enzymatic therapy combined with pneumatic displacement by intravitreally injected gas. Increasing experience with pars plana vitrectomy demonstrated that a complete removal of the vitreous body has beneficial effects on the course of vasoproliferative vitreoretinal diseases. Therefore enzymes were tested to either liquify the vitreous body (collagenase or hyaluronidase) or to cleave the posterior vitreous cortex and the retina (dispase, plasmin, tissue plasminogen-activator or chondroitinase). At present only tissue-plasminogen activator (TPA), plasmin and hyaluronidase were used in small clinical studies. Recent developments in the understanding of vasoproliferative vitreoretinal disorders offers new therapeutical approaches like enzymatical destruction of growth factors (VEGF) or extracellular adhesive proteins (fibronectin). From this point of view future therapies may include enzymatic cleaning of the vitreous body to prevent proliferative diabetic vitreoretinopathy.

Keywords

Vitreous · Enzyme · Fibrinolysis · Plasmin · Tissue plasminogen activator

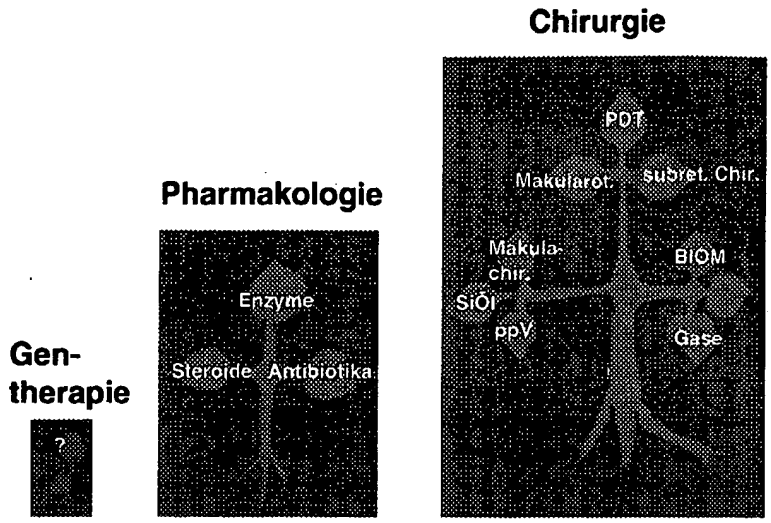

Abb. 1 ▲ **Grafische Darstellung derzeitiger therapeutischer Optionen vitreoretinaler Erkrankungen**

kleine durchgreifende Netzhautdefekte, die eine Passage von TPA durch die Netzhaut ermöglichen. Durch subretinale Injektion von Tinte konnte diese These kürzlich im Tiermodell bestätigt werden [3]. Die Injektion kleiner Volumina induzierte eine umschriebene Abhebung der Netzhaut, aber erst bei größeren Volumina perforierte die Netzhaut und färbte den angrenzenden Glaskörper. Das therapeutische Prinzip basiert somit auf einem Synergismus aus enzymatischer Verflüssigung und mechanischer Verdrängung des subretinalen Koagels. Zwar mag die Verdrängung auch ohne vorherige Verflüssigung im Einzelfall gelingen [21], aber eben weniger ausgeprägt [9].

Retinale Toxizität von TPA?

Gegenwärtig ist die Frage der retinalen Toxizität von injiziertem Gewebeplasminogenaktivator noch nicht abschließend geklärt. Nach intravitrealer Injektion von TPA gelangen bisher nicht genauer quantifizierte Mengen des Enzyms in den subretinalen Raum. Unstrittig sind toxische Effekte nach Injektion von 100 µg TPA beim Menschen (exsudative Netzhautablösungen, nachfolgende Pigmentveränderungen des RPE, ERG-Veränderungen; [13]) und im Tierversuch (Retinanekrosen, ERG-Veränderungen) beobachtet worden [15, 16]. Die Autoren folgerten aus ihren Ergebnissen, dass eine „Injektion von mehr als 25 µg TPA beim Menschen möglicherweise nicht sicher sei" [15]. Grundsätzlich ist der Vorschlag einer niedrigeren Dosierung sinnvoll, aber gegenwärtig fehlt der Nachweis, dass diese geringe Menge für eine subretinale Fibrinolyse ausreichend ist. Falls eine Dosis von 25 µg nur eine unvollständige Verflüssigung und damit eine unvollständige Verlagerung des Koagels bewirkt, so werden massive irreparable Netzhautschäden durch den Abbau der Blutung entste-

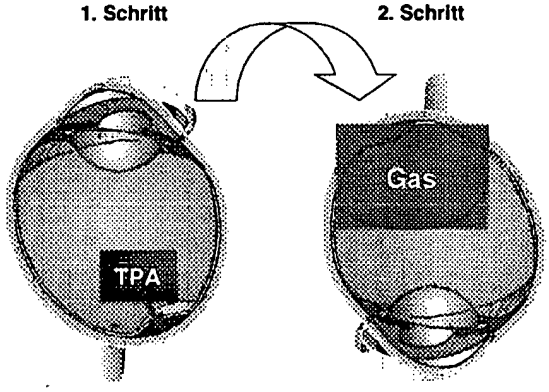

Abb. 2 ◀ **Prinzip der enzymatisch-mechanischen Therapie zur Behandlung subretinaler Blutungen. Zunächst diffundiert intravitreal injizierter Gewebeplasminogenaktivator (TPA) durch die Netzhaut in das Koagel und induziert dort eine Fibrinolyse. Das verflüssigte Blut wird durch ein nachfolgend injiziertes expansives Gas bei entsprechender Kopfpositionierung aus der Makula nach unten verdrängt**

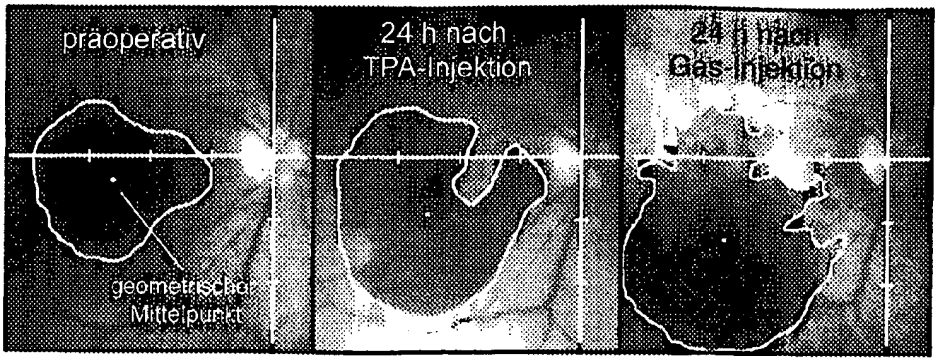

Abb. 3 ◀ **Prinzip der morphometrischen Analyse. Fläche und geometrischer Mittelpunkt der subretinalen Blutung wurde vor und einen Tag nach TPA-Injektion bzw. einen Tag nach Gasinjektion bestimmt. Nach der TPA-Injektion ist bereits eine deutliche Vergrößerung der Fläche als Zeichen einer Verflüssigung des Koagels erkennbar. Durch die Gasblase wird verflüssigtes Blut vorwiegend nach unten gedrängt**

hen [31]. Zwei weitere Gesichtspunkte wurden in diesem Zusammenhang diskutiert. Zum einen ist das Glaskörpervolumen der Katze erheblich geringer verglichen zum Menschen, weswegen eine Dosierung von 25 µg bei der Katze einer Dosis von 36 µg beim Menschen entspricht. Zum anderen ist der Glaskörper der älteren Patienten regelmäßig verflüssigt, wodurch sich TPA im gesamten Glaskörper verteilen kann und nicht wie bei der Katze nahe der Injektionsstelle konzentriert bleibt. Schließlich kann eine mögliche toxische Schädigung durch TPA vermindert werden, wenn auf eine gleichzeitige Injektion von Gas verzichtet wird [11]. Expandierende Gase verdoppeln ihr Volumen innerhalb von 6 h nach Injektion und erhöhen damit die Konzentration von TPA in den verbliebenen Anteilen des Glaskörpers. Wird dagegen die Gasinjektion 12 oder 24 h später vorgenommen, so ist bereits die Hälfte bzw. Dreiviertel des TPAs verschwunden. Eine retinatoxische Wirkung von TPA ist klinisch an Patienten schwierig nachzuweisen, da sie von Netzhautveränderungen verursacht durch subretinales Blut oder eine zugrunde liegende altersbedingte Makuladegeneration praktisch kaum abgegrenzt werden kann. Daher sind gegenwärtig auch keine Studiendaten verfügbar, die belegen könnten, dass weniger als 50 µg TPA injiziert werden sollten, um eine toxische Wirkung von TPA an der Netzhaut zu vermeiden.

Enzymatische Behandlung der proliferativen diabetischen Vitreoretinopathie

Derzeit sind 4 unterschiedliche Ansätze zur enzymatischen Behandlung dieser Erkrankung vorstellbar (Tabelle 1), die im Folgenden vor dem pathophysiologischen Hintergrund erläutert werden. Der proliferative Verlauf der diabetischen Retinopathie beginnt mit Aussprossung von Neovaskularisationen aus der Netzhaut in den Glaskörper. Dabei nutzen wachsende Zellen den Glaskörperkortex als Leitstruktur. Wenn diese Leitstruktur fehlt, weil eine vollständige hintere Glaskörperabhebung besteht, kann kein proliferatives Stadium der Retinopathie entstehen, es wurde sogar eine Rückbildung proliferierender Gefäße und Membranen bei vollständig abgehobenem Glaskörper beobachtet [26, 29]. Dagegen begünstigt ein nur partiell abgehobenem Glaskörper eine rasche Progredienz des proliferativen Prozesses [2], da mechanische Spannungen auf Zellen einen stimulierenden Effekt haben. Derzeit lässt sich eine hintere Glaskörperabhebung verlässlich nur chirurgisch durch eine Pars-plana-Vitrektomie erzeugen. Zwar kann auch durch eine panretinale Laserkoagulation eine Abhebung des Glaskörpers induziert werden [18], doch ist diese Methode nicht zuverlässig, weil häufig der Glaskörperkortex lediglich gespalten (Vitreoschisis) wird und periphere Anteile des Glaskörperkortex auf der Netzhaut haften bleiben [24].

Tabelle 1
Zusammenfassung der therapeutischen Ziele und bisher untersuchter Substanzen zur enzymatischen Behandlung einer proliferativen diabetischen Vitreoretinopathie

Therapeutisches Ziel	Eingesetztes Enzym	Untersuchungen	
		In vitro	In vivo
Hintere Glaskörperabhebung	Plasmin	Ja	Ja
	TPA	Nein	Ja
	Dispase	Ja	Nein
	Chondroitinase	Ja	Ja
	Hyaluronidase	Ja	Ja
Verflüssigung des Glaskörpers	Hyaluronidase	Ja	Ja
Proteolyse von VEGF	Plasmin	Ja	Nein
Generierung von Angiostatin	Plasmin und Reduktasen	Ja	Nein
	MMP 7 und MMP 9	Ja	Nein

TPA *Gewebeplasminogenaktivator*, MMP *Metalloprotease*, VEGF *„vascular endothelial growth factor"*.

Enzymatische Abhebung des Glaskörpers

Eine enzymatische Abhebung des Glaskörpers wäre also eine wünschenswerte therapeutische Option. Leider ist bisher nicht genau bekannt, welche molekularen Strukturen für die Adhäsion des Glaskörpers an der Lamina limitans interna der Netzhaut verantwortlich sind und enzymatisch zerstört werden müssten. Gegenwärtige Vorstellungen zum Aufbau des vitreoretinalen Interfaces sind in Abb. 4 zusammengefasst. Trotzdem gelang die enzymatische Abhebung des Glaskörpers tierexperimentell bisher mit mehreren

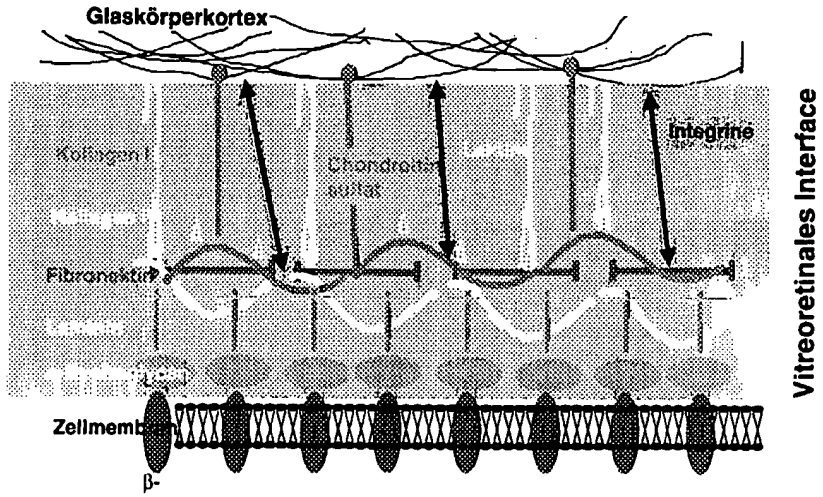

Abb. 4 ▲ Schematische Darstellungen zum Aufbau des vitreoretinalen Interfaces als Verbindungselement zwischen Netzhaut und Glaskörper. Hauptelemente sind die Matrixproteine Laminin, Fibronektin und Kollagen IV, die durch Dystroglykane über die Zellmembran mit den Müller-Zellen verankert sind. Als Linker-Moleküle zum Glaskörper werden Chondroitine, Lektine, Integrine oder spezifische Bindungsdomänen der Matrixproteine diskutiert

Proteasen wie Plasmin, Dispase, Chondroitinase und Gewebeplasminogenaktivator [6, 7, 12, 30, 33]. Bisher wurden lediglich Gewebeplasminogenaktivator und Plasmin, beides Serinproteasen des fibrinolytischen Systems, beim Menschen angewandt [10, 32].

Enzymatische Verflüssigung des Glaskörpers

Eine zweite Möglichkeit zur Induzierung einer hinteren Glaskörperabhebung ist die enzymatische Verflüssigung des Glaskörpers (Syneresis). Normalerweise ist die Syneresis ein altersabhängiger Prozess, der bei mehr als 80% der über 60-Jährigen zu finden ist [20]. Zerfällt die dreidimensionalen Gelstruktur, so entstehen charakteristische fibrillenarme Lakunen und kondensierte Fibrillenstränge. Ständige Augenbewegungen bewirken eine Volumenverschiebung, die sukzessive zur vollständigen hinteren Glaskörperabhebung führt [25]. Eine Verflüssigung des Glaskörpers kann sehr einfach durch Injektion von Hyaluronidase in den Glaskörper erzielt werden. Dieses hochspezifische Enzym spaltet ausschließlich Hyaluronsäure und hat daher keine toxischen Eigenschaften. Die Verflüssigung des Glaskörpers ist gegenwärtig Ziel der enzymatischen Behandlung einer Glaskörperblutung im Rahmen einer randomisierten prospektiven Multicenterstudie. Der intakte Glaskörper behindert die spontane Aufklarung einer Blutung, weil zelluläre Blutbestandteile zwischen den Glaskörperfasern wie in einem Netz gefangen werden (Abb. 5). Allerdings ist die Verflüssigung des Glaskörpers nicht ungefährlich, wenn gleichzeitig vitreoretinale Adhärenzen bestehen, die unter dem eingebluteten Glaskörper nicht kontrolliert werden können.

Proteolyse von Wachstumsfaktoren oder extrazellulären Matrixproteinen

Durch zellbiologische Erkenntnisse aus den letzten Jahren zur Rolle von Wachstumsfaktoren in der Pathophysiologie vasoproliferativer vitreoretinaler Erkrankungen ergeben sich heute neue therapeutische Optionen. Das Ziel einer zukünftigen Therapie der proliferativen diabetischen Vitreoretinopathie könnte die Proteolyse von Wachstumsfaktoren oder extrazellulären Matrixproteinen (z. B. Fibronektin) sein. So konnte die mitogene Wirkung von VEGF („vascular endothelial growth factor"), dem stärksten bekannten angiogenetischen Wachstumsfaktor, nachgewiesen in Netzhaut und Glaskörper, in vitro durch Spaltung mit Plasmin hundertfach verringert werden (Abb. 6) [17]. Denkbar wäre die Injektion von Plasmin in den diabetischen Glaskörper, um VEGF im Auge zu vermindern, ähnlich wie dies derzeit mit einer panretinalen Laserkoagulation bewirkt werden kann [1].

Behandlung durch Angiogeneseinhibitoren

Als derzeit letzte Option wäre die Behandlung durch geeignete Angiogeneseinhibitoren zu nennen. Einer der stärksten Hemmer der Angiogenese ist das Angiostatin [22], das aus den Kringeln 1–3 des Plasminogens besteht. Derzeit sind die Bedingungen und Enzyme, durch die Plasminogen oder Plasmin in den hochwirksamen Angiogenesinhibitor gespalten wird, nicht genau bekannt [23, 27, 28]. Wenn diese Stoffwechselwege geklärt sind, würde sich der diabetische Glaskörper idealerweise für eine enzymatische Therapie anbieten. Bei der diabetischen Retinopathie treten Plasmaproteine einschließlich Plasminogen [4] in den Glaskörper über infolge einer lang dauernden Störung der Blut-Retina-Schranke. Wenn es gelänge intravitreales Plasminogen in vivo in das hochwirksame Angiostatin enzymatisch zu spalten, könnte der Glaskörper der Patienten mit Diabetes mellitus die Funktion eines „drug-delivery systems" übernehmen.

Abb. 5 ▶ Rasterelektronenmikroskopische Aufnahme einer Glaskörperblutung. Erythrozyten sind in einem Netzwerk von Fibrin und kollagenen Fasern immobilisiert

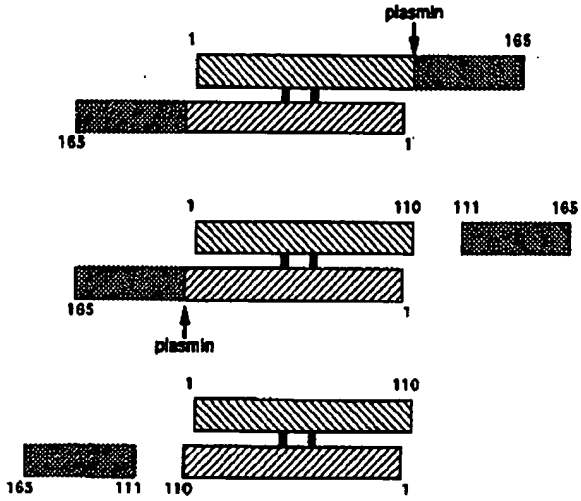

Abb. 6 ◀ **Schematische Darstellung der enzymatischen Spaltung von VEGF („vascular endothelial growth factor") durch Plasmin [17]. Nach enzymatischer Spaltung durch Plasmin verringert sich die biologische Wirksamkeit des verbliebenen Fragments um mehr als das hundertfache gegenüber dem vollständigen VEGF**

Fazit für die Praxis

Zurzeit gewinnt die enzymatische Behandlung von Erkrankungen im hinteren Augenabschnitt zunehmend an Bedeutung. Morphometrische Analysen konnten eine subretinale Fibrinolyse nach intravitrealer Injektion von Gewebeplasminogenaktivator nachweisen. Damit kann erstmals eine aufwendige Pars-plana-Vitrektomie durch ein enzymatisches Therapieverfahren ersetzt werden.
Das derzeitige pathophysiologische Verständnis der diabetischen Retinopathie bietet mehrere Ansatzpunkte zur Behandlung eines proliferativen Verlaufs, was eine zukünftige Behandlung des diabetischen Glaskörpers mit geeigneten Enzymen greifbarer werden lässt.

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request's status [redacted]

Jacob, Re ca (ASRC) 122797

From: STIC-ILL
Sent: Wednesday, December 04, 2002 10:58 AM
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Subject: FW: Articles

-----Original Message-----
From: Solomon, Terrance
Sent: Wednesday, December 04, 2002 10:57 AM
To: STIC-ILL (E-mail)
Cc: DeSanto, Matthew
Subject: Articles

The following articles **were not** available full-text using STIC resources:

Ophthalmology
Vol-108
Issue-10
" Autologous Plasmin Enzyme in the Surgical Management of Diabetic Retinopathy"
Williams, J.G.; et al
Pages:1902-1905
Year-2001

Seminars in Ophthalmology
Vol-15
Issue-2
" Enzymatic Vitreous Surgery"
Trese, M.T.
Pages:116-121
Year-2000

For **Matthew F. DeSanto, AU 3763, 305-3292, US serial NOT GIVEN.**

Thanks,

Terry Solomon
EIC 3700
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CP2, 2C08

Autologous Plasmin Enzyme in the Surgical Management of Diabetic Retinopathy

Jonathan G. Williams, MD,[1] Michael T. Trese, MD,[1,2] George A. Williams, MD,[1,2] Michael K. Hartzer, PhD[2]

Purpose: This is a pilot study to assess the use of autologous plasmin enzyme (APE) as an adjunct to vitreous surgery in eyes with advanced diabetic retinopathy.

Design: Prospective noncomparative interventional case series.

Participants: Seven patients with advanced diabetic retinopathy selected at random from our practice population.

Methods: Seven eyes were treated with APE as an adjunct to standard vitreous surgery. Six eyes had macular tractional retinal detachments, and one eye had refractory macular edema. Three fellow eyes had standard vitreous surgery performed for macular tractional retinal detachments without APE. All 10 eyes had macular edema and background diabetic retinopathy.

Main Outcome Measures: The main outcome measures included induction of a posterior vitreous detachment, retinal reattachment, improvement in visual acuity, and resolution of macular edema.

Results: All seven APE-treated eyes achieved spontaneous or easy removal of the posterior hyaloid including one eye that had vitreoschisis over areas of detached retina. All eyes treated with APE had resolution of intraretinal edema. Retinas of all eyes treated with APE were reattached. The three fellow eyes were treated by vitreous surgery without APE. Two of the three fellow eyes had reattached retinas, but none had resolution of intraretinal edema without further focal photocoagulation treatment. Mean visual acuity improvement was 0.7 logarithm of the minimum angle of resolution (LogMAR) units in APE-treated eyes and 0.1 LogMAR units in eyes without APE. The average follow-up period was 14 months.

Conclusions: This pilot study suggests that APE may be beneficial in the surgical management of diabetic retinopathy. *Ophthalmology 2001;108:1902–1905 © 2001 by the American Academy of Ophthalmology.*

Traction at the vitreoretinal interface is a major cause of visual morbidity in diabetic retinopathy. It may result in tractional retinal detachment and/or intractable diabetic macular edema that is refractory to laser treatment.[1–3] Despite major advances in vitreoretinal surgical techniques over the past 30 years, relief of vitreoretinal traction remains the most critical and perhaps the most difficult step in the surgical management of diabetic retinopathy.[4–7] Removal of the posterior hyaloid in diabetic tractional retinal detachments can be particularly challenging, because tractionally elevated retina is often diaphanous, atrophic, and difficult to distinguish from cortical vitreous and epiretinal membranes. Tissue plane identification can further be complicated by the increased incidence of vitreoschisis in diabetic retinopathy.[8,9]

We have previously demonstrated several applications of autologous plasmin enzyme (APE) in the treatment of ocular disease. Plasmin can create a posterior vitreous detachment (dose-dependent) in the rabbit and pig[10–12]; 0.4 IU of plasmin can induce posterior vitreous detachments (PVDs) in humans, facilitating membrane peeling in complicated vitreoretinal surgery and treating pediatric macular holes and idiopathic stage 3 macular holes in adults.[13–15] The enzymatic effect of plasmin in these applications seems to be attributable to the proteolysis of laminin and fibronectin, both of which are present at the vitreoretinal interface.

In this pilot study, we investigated APE in the surgical management of diabetic retinopathy by assessing intraoperative spontaneous PVD or the subjective ease with which the posterior hyaloid separated from the retina, postoperative retinal reattachment, resolution of macular edema, and visual improvement.

Methods and Patients

After institutional review board approval, a separate informed consent for the use of APE was obtained for each patient for this study. APE was prepared as previously described.[13] Autologous blood was drawn before surgery, and plasminogen was isolated from human plasma by affinity chromatography on a lysine-Sepharose column. The plasminogen was eluted using 15 mM epsilon aminocaproic acid, and the aminocaproic acid was then removed by overnight dialysis. The plasminogen was concentrated to a volume of 1 ml and activated by the addition of 50,000 IU of streptokinase followed by sterilization through a 0.22-μm filter.

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Michael T. Trese, MD, George A. Williams, MD, and Michael K. Hartzer, PhD, have a proprietary interest in the use of autologous plasmin enzyme in intraocular surgery.

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Plasmin activity was determined spectrophotometrically by measuring the change in absorbance at 405 nm after cleavage of a D-val-leu-lys-*p*-nitroaniline substrate spectrophotometrically. Plasmin was stored at 4° C until used.

Seven patients with advanced diabetic retinopathy were selected at random from the group of diabetic patients seen in our practice. One eye had refractory macular edema and a small neovascular frond at the disc. Six eyes had macular tractional retinal detachments. No patient had clinical evidence of a PVD or vitreoschisis preoperatively. Four patients were male, and three were female. All patients were white. Their ages ranged from 40 to 75 years, with a mean age of 55.7 years. In three patients, the fellow eyes had previously received standard vitreous surgery without APE. An effort was made to match the pathology in the fellow eyes to the APE-treated eyes as closely as possible to allow comparison.

Fifteen minutes before surgical incision, 0.4 IU of APE in a volume of 0.1 ml was injected into the midvitreous. Our previous research indicates that maximal plasmin enzymatic activity occurs 15 to 60 minutes after injection.[10] The patient was then prepared and draped, and vitreous surgery was carried out by two-port pars plana vitrectomy technique using an infusion/fiberoptic light source and vitreous cutter. The eyes were entered and, before the beginning of vitrectomy, the status of the posterior hyaloid was determined, specifically whether it was attached or detached in areas without cellular attachments to the retina. If the hyaloid had not separated spontaneously, suction was used with the vitreous cutter to remove the hyaloid from the anterior retinal surface. When suction was needed, never more than the 50 mmHg for longer than 30 seconds was required. In the six cases of tractional retinal detachment treated with APE, dissection of "pegs" along the posterior pole of vascularized cellular tissue was necessary. In the one case of refractory macular edema, no peeling along the posterior pole was performed. After dissection as needed, a fluid–air exchange was performed in all eyes with or without the use of APE, although no retinal tears were present. Endolaser was performed in four eyes that had APE and in all three eyes with standard vitreous surgery to fill in panretinal photocoagulation. No panretinal photocoagulation was done in the eye operated on for refractory macular edema with a small neovascular frond at the disc. In no eye was focal laser performed.

Subconjunctival injections of gentamicin and dexamethasone were administered immediately postoperatively in eyes in which APE had been used. Topical erythromycin ointment was then applied, and patching was performed. Patients who did not have APE had standard diabetic vitreous surgery techniques identical to the eyes in which enzyme had been used. Care was taken to evaluate the ease with which the hyaloid could be peeled, both in areas of attached and detached retina.

Patients were examined at 1 day, 1 week, 2 weeks, and then monthly after surgery. The average follow-up period was 14 months (13 months for the APE group and 19 months for the fellow eyes). Examinations included best-corrected visual acuity, applanation tonometry, slit-lamp examination, and indirect ophthalmoscopy.

Results

Six of six eyes (100%) treated with APE for tractional retinal detachments showed retinal reattachment, whereas two of three fellow eyes (67%) showed retinal reattachment. The one eye treated with APE for refractory macular edema had a small neovascular frond at the disc. This edema and frond showed resolution postoperatively, and the retina remained attached (Fig 1A, B). In addition, the six eyes treated for tractional retinal detachment that had diabetic macular edema and background diabetic retinopathy before surgery showed resolution of diabetic macular edema with no eye needing focal laser treatment postoperatively. None of the fellow eyes had resolution of macular edema without further focal laser treatment.

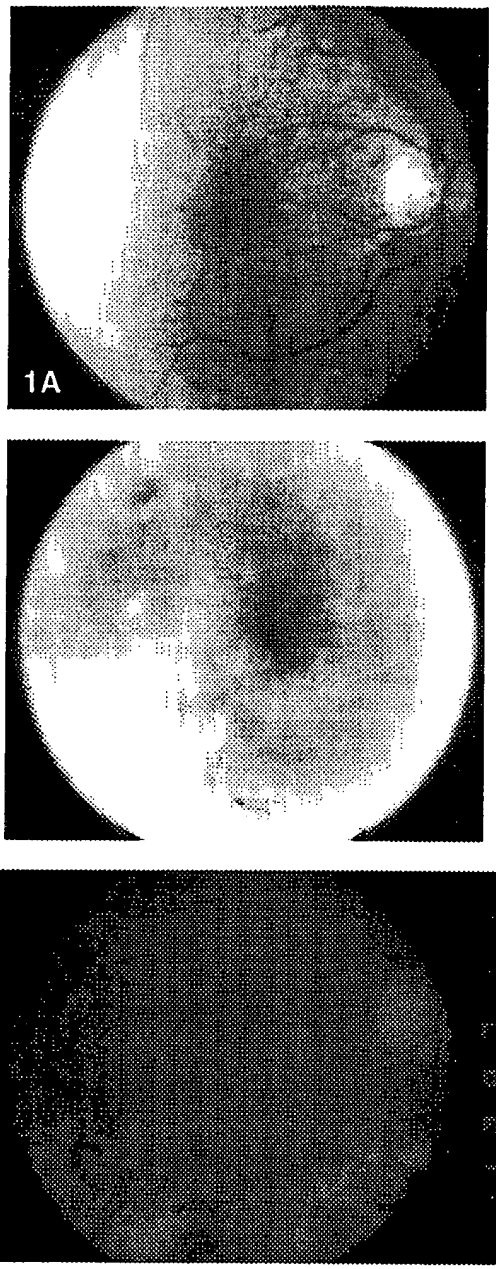

Figure 1. A, Preoperative angiogram of patient who had refractory macular edema and a peripapillary frond. B, Postoperative fundus photograph showing resolution of neovascularization and macular edema.

In all of the APE-treated eyes, the vitreous over retina without cellular proliferation showed either spontaneous or much easier peeling, allowing the surgeon to easily peel the outer layer of a vitreoschisis cavity from the detached retina where vitreoschisis had not been observed during the preoperative examination. Five eyes demonstrated spontaneous PVDs, whereas PVDs were achieved in the other two eyes with minimal peeling (Table 1).

Table 1. Patient Data Table

Patient	Age	Gender	Eye	Preoperative Diagnosis	Procedure	CSME Preoperative-Postoperative	Visual Acuity Preoperative-Postoperative	Posterior vitreous Detachtment*
1 MC	62	F	OS	PDR, TRD, VH	APE, Vx, MP, FAX	Yes/No	1/200; 20/800	Easy to peel
	62	F	OD	PDR, TRD, NVI	Vx, MP, EL, FAX	Yes/Yes	HM; NLP	NA
2 DA	40	M	OD	PDR, TRD	APE, Vx, MP, FAX	Yes/No	20/200; 20/30	Spontaneous
	40	M	OS	PDR, TRD	Vx, MP, EL	Yes/Yes	20/60; 20/60	NA
3 MP	75	M	OD	PDR, TRD	APE, Vx, MP, EL, FAX	Yes/No	20/200; 20/40	Spontaneous
	75	M	OS	PDR, TRD, VH	Vx, MP, EL	Yes/Yes	20/200; 20/80	NA
4 BS	47	F	OD	PDR, TRD, VH	APE, Vx, MP, EL, FAX	Yes/No	20/40; 20/20	Spontaneous
5 DG	43	M	OS	PDR, TRD, VH	APE, Vx, MP, EL, FAX	Yes/No	20/400; 20/50	Easy to peel
6 WB	66	M	OD	BDR, CSME	APE, Vx, MP	Yes/No	20/200; 20/30	Spontaneous
7 MN	57	F	OD	PDR, TRD, VH	APE, Vx, MP, EL, FAX	Yes/No	5/200; 20/40	Spontaneous

APE = autologous plasmin enzyme; BDR = background diabetic retinopathy; CSME = clinically significant macular edema; EL = endolaser; FAX = fluid air exchange; HM = hand motions; MP = membrane peeling; NLP = no light perception; NVI = iris neovascularization; OD = right eye; OS = left eye; PDR = proliferative diabetic retinopathy; TRD = tractional retinal detachment; VH = vitreous hemorrhage; Vx = vitrectomy.

*Posterior vitreous detachment in area without epiretinal cellular tissues.

This is noteworthy in that it represents a critical step in the surgical management of diabetic retinopathy, and its spontaneous occurrence is exceedingly uncommon. Posterior vitreous separation was determined subjectively by the same surgeon (MTT) without the aid of ultrasonography or OCT. The effect of APE at the vitreous base was not assessed.

Mean visual acuity improvement was 0.7 logarithm of the minimum angle of resolution units (logMAR) for APE-treated eyes and 0.1 logMAR without APE (see Table 1 for Snellen equivalents). Visual acuity was measured in a masked fashion using best-corrected visual acuity with an autorefractor. It is interesting that the maximum visual acuity was achieved more rapidly in the eyes treated with APE than in eyes treated with standard vitreous surgery. The APE-treated eyes also achieved a better final visual acuity. In patient 2, the APE-treated eye achieved 20/30 vision after 3 weeks, and the conventionally treated eye achieved 20/60 vision after 4 months and one focal laser treatment. For patient 3, the APE-treated eye achieved 20/40 vision after 10 months, and the conventionally treated eye achieved 20/80 vision after 27 months and three focal laser treatments. In the third patient on whom a fellow eye was operated (patient's), one eye had very severe retinal ischemia and neovascular glaucoma developed and progressed to no light perception in the eye treated without APE. This patient's other eye, also with severe ischemia, underwent APE-assisted vitrectomy and improved to only 20/800 visual acuity.

No complications occurred with the use of APE in this cohort of patients. Specifically, no postoperative rise in intraocular pressure was seen. In addition, no evidence of intraretinal or vitreous bleeding was found. Postoperative anterior segment inflammation was minimal and was consistent with the inflammation seen in the fellow eyes.

Discussion

We believe that APE may be helpful in the management of diabetic retinopathy during vitreous surgery in two ways. One, APE facilitates membrane peeling by acting on the extracellular matrix fibrin between the cellular pegs of the epiretinal membrane, allowing better exposure of the pegs and making them easier to divide. Two, APE stimulates spontaneous or easier-to-peel posterior hyaloid where cellular proliferation is not present. The complete, less traumatic, enzymatically assisted removal of epiretinal tissue, particularly vitreous cortex in the macular area, we believe, may lead to more rapid resolution of intraretinal edema. This enzymatic approach may eliminate iatrogenic vitreoschisis in the macular area during mechanical membrane peeling, which can leave the surgeon with the false impression that the complete posterior hyaloid has been peeled.

It is this complete posterior hyaloid removal that we believe allows complete resolution of macular edema. These remnants of posterior hyaloid on the macular area may have contributed to the persistence of macular edema and led to the need for focal laser after vitrectomy in the eyes treated by standard technique. The visual improvement of 0.7 logMAR units in the APE-treated eyes represents a sevenfold relative improvement over the fellow eyes. This is clinically significant in that it represents a 233% decrease in the visual angle (an improvement of 0.3 logMAR units equates to halving of the visual angle). Although neither group showed iatrogenic retinal tear, it might be that this enzymatic membrane peeling is a less traumatic approach to PVD and may lead to fewer tears.

The authors are unable to draw any broad conclusion from their pilot study data, but they do suggest that a larger study using APE or other adjuncts aiding in atraumatic or spontaneous PVD may be helpful in vitreous surgery. Such a study is planned. It is interesting that the eyes treated with APE did not require additional focal laser to achieve maximum visual acuity, and the patient with a small peripapillary frond and refractory clinically significant macular edema did not even need mechanical membrane peeling to show resolution of neovascularization and macular edema. We believe this suggests that a cleaner cleavage plane is achieved with enzyme-assisted membrane peeling and that mechanical membrane peeling may create vitreoschisis at the time of conventional vitreous surgery. The use of other magnification aids or vital dyes or even OCT (not available during this study) may be helpful in the future to identify

whether cortical vitreous collagen is left after conventional membrane peeling.

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Discussion

by
Peter Kroll, MD, Lutz Hesse, MD

Over time, numerous treatment modalities have been replaced by less invasive procedures. As an example, subretinal hemorrhages are now treated by an intravitreal injection of tissue plasminogen activator and gas instead of mechanical extraction of the clot by a pars plana vitrectomy.[1–3]

The authors have presented their experience treating proliferative diabetic vitreoretinopathy (PDVR) by intravitreal injection of autologous plasmin enzyme (APE) before vitrectomy. In this disease the vitreous cortex acts as a metabolic barrier and as a scaffold for proliferating cells.[4] Late stages of the disease result from vitreous contractions and subsequent tractional-rhegmatogenous detachment. The rationale of an enzymatic treatment in PDVR is to detach the posterior vitreous cortex. Six patients with a mean follow-up period of 14 months were included. Posterior vitreous detachment was achieved in all APE-treated eyes.

In the past, several enzymes have been used to induce a posterior vitreous detachment. Hyaluronidase, collagenase, chondroitinase, dispase, plasmin and tissue plasminogen activator (tPA) were tested in animals (Hageman GS, Russell SR, Invest Ophthalmol Vis Sci 1994;35:1260)[5–8] but only plasmin and tPA, both enzymes of the fibrinolytic process, were applied in humans with more or less success.[9–13]

The mechanism of plasmin-induced posterior vitreous detachment (PVD) is not completely understood. The internal limiting membrane (ILM) is composed mainly of type-IV collagen, fibronectin, and laminin.[14] At present, it is not known which components of the ILM must be dissolved by plasmin to provoke a PVD. Possibly "linker molecules" like lectins, integrins, and chondroitin sulfate responsible for the attachment of the vitreous cortex to the ILM may be the target of an unspecific protease like plasmin.

The use of an enzyme immediately before vitrectomy is the most comfortable technique for both patient and surgeon. Plasmin acts immediately after injection, whereas tPA needs some hours to generate plasmin from its precursor plasminogen.[10,15] From these results, we concluded that tPA, in contrast to plasmin, cannot be applied at the beginning of pars plana vitrectomy. This seems to be the significant advantage of plasmin in contrast to tPA. For the resolution of a diabetic macular edema in APE-treated eyes in contrast to non-APE-treated eyes, we still do not have any explanation. In many articles, a regression of diabetic macular edema was observed after removing the posterior vitreous cortex[16,17] and even the ILM.[18] On the basis of our current knowledge, it remains unclear why, after vitrectomy, the macular edema only disappeared in the APE-treated eyes and not as well in the non-APE-treated eyes where a complete vitrectomy had also been performed. A reason could be that remnants of a vitreoschisis in the non-APE-treated eyes were not removed completely during vitrectomy. This may also be the reason for the development of a redetachment in one of the three non-APE-treated eyes. Our current high-tech mechanical instrumentation is wonderful, but the future will most likely belong to biologic treatment modalities like the application of enzymes. In the future surgical intervention in macular holes, macular edema, PVR, PDVR and other disorders may be obsolete, as well as many other of our current indications for pars plana vitrectomy. Particularly in the prevention of many vitreoretinal diseases, enzymatic applications may be helpful to circumvent further surgery.

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Jacob, Rebecca (ASRC) 422816

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Seminars in Ophthalmology
Vol-15
Issue-1
" Tissue Plasminogens Activator in the Treatment of Vitreoretinal Diseases"
Kamei, M.; Estafanous, M.; Lewis, H.
Pages:44-50
Year-2000

Retina
Vol-19
Issue-1
" Posterior Vitreous Detachment Induced by Injection of Plasmin and Sulfur Hexafluoride in the Rabbit Vitreous"
Hikichi, T.; et al
Pages:55-58
Year-1999

For **Matthew F. DeSanto, AU 3763, 305-3292, US serial NOT GIVEN**.

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Terry Solomon
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POSTERIOR VITREOUS DETACHMENT INDUCED BY INJECTION OF PLASMIN AND SULFUR HEXAFLUORIDE IN THE RABBIT VITREOUS

TAIICHI HIKICHI, MD, NORIHIKO YANAGIYA, MD, MASANORI KADO, MD, JUN AKIBA, MD, AKITOSHI YOSHIDA, MD

Purpose: To investigate whether an injection of plasmin and sulfur hexafluoride (SF_6) can induce posterior vitreous detachment (PVD) without vitrectomy.

Methods: One eye each of 15 New Zealand white rabbits was assigned to one of three groups. Eyes in group 1 received a vitreous injection of 1 unit of human plasmin (0.1 mL reconstituted in balanced salt solution) and 0.5 mL of SF_6; eyes in group 2 received a vitreous injection of plasmin alone; eyes in group 3 received a vitreous injection of SF_6 alone. Seven days after injection, all animals were monitored electroretinographically and killed, and the eyes were enucleated. After fixation, scanning electron microscopy was performed.

Results: In group 1 eyes, the retinal surface was smooth except for the vitreous base, which showed complete separation of the vitreous cortex from the retina, indicating PVD. In group 2 and 3 eyes, sparse collagen fibers remained on the retinal surface.

Conclusion: Vitreous injection of plasmin combined with SF_6 can induce PVD without vitrectomy.

RETINA 19:55–58, 1999

Two important goals of vitreous surgery are separation of the vitreous from the retina and release of vitreous traction. Especially in some retinal diseases such as macular holes[1] or diabetic macular edema,[2] removal of the vitreous cortex from the retina is the primary surgical maneuver. However, surgical removal of the vitreous cortex is sometimes difficult and carries the risk of complications such as retinal breaks, retinal detachment, or retinal nerve fiber damage.[3,4]

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The authors have no proprietary interest in any aspect of this study.

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In the natural history of some retinal disorders, the role of vitreoretinal adhesion is well established. Complete posterior vitreous detachment (PVD) may prevent retinal neovascularization in eyes with diabetic retinopathy and retinal vein occlusion.[5,6] The prognosis of proliferative diabetic retinopathy is much better in eyes with complete PVD than in eyes with no or partial PVD.[7] Vitreous traction on the fovea is a major cause of idiopathic macular holes, and vitreous separation from the fovea may greatly reduce the risk of macular hole formation.[8]

Verstraeten and associates[9] demonstrated that plasmin could be a useful biochemical adjunct to mechanical vitrectomy and facilitate PVD formation. Separating the vitreous cortex from the retina without vitrectomy would be more valuable clinically. In the current study, to investigate the possibility of PVD

formation without vitrectomy, we evaluated if vitreous injection of plasmin and sulfur hexafluoride (SF_6) could induce PVD without vitrectomy.

Materials and Methods

Preparation of Plasmin Solution

Plasmin (CalBiochem, La Jolla, CA) was stored at −20°C until administration, at which time the powder was reconstituted in sterile balanced salt solution (BSS) at room temperature to a final concentration of 1 unit of 0.1 mL.[9]

Injection of Plasmin and SF_6

Fifteen male New Zealand white rabbits (2–2.5 kg) were housed in the animal care facilities of Asahikawa Medical College, Asahikawa, Japan. The animals were fed standard laboratory chow and treated in accordance with the Principles of Laboratory Animal Care of the National Institutes of Health. Rabbits were anesthetized with a combination of intramuscularly injected ketamine hydrochloride (20 mg/kg) and chlorpromazine hydrochloride (10 mg/kg) and topical proparacaine. Five rabbits each were randomly assigned to one of three groups. One eye of each rabbit in group 1 received a pars plana injection in the midvitreous cavity of 1 unit of human plasmin (0.1 mL reconstituted in sterile BSS) and 0.5 mL of SF_6. One eye of each rabbit in group 2 received a vitreous injection of 1 unit of human plasmin (0.1 mL) only. One eye of each rabbit in group 3 received a vitreous injection of 0.5 mL of SF_6 only.

The fellow eye of each rabbit was injected with BSS (0.1 mL).

Clinical Examination

Before the study and at periodic intervals after intravitreal injection, indirect ophthalmoscopic and slit-lamp biomicroscopic examinations with and without a preset lens (−90 diopters, Nikon, Tokyo, Japan) were performed. We also monitored intraocular pressure (IOP) levels of the injected eyes using a calibrated pneumotonometer (Model 30 Classic Pneumotonometer, Mentor O & O, Norwell, MA) after application of one drop of 0.4% oxybuprocaine hydrochloride at baseline and periodic intervals after intravitreal injection, and confirmed that the IOP did not increase. The mean IOP of three measurements was calculated at each time point. When the IOP rose after intravitreal injection of SF_6, paracentesis was performed and the IOP was decreased to the preinjection level.

Electrophysiologic Examination

Seven days after intervention, electroretinography (ERG) was performed in all rabbits. The rabbits were anesthetized with 10 mg/kg intravenous pentobarbital sodium, the pupils were dilated, and topical anesthetic was applied to the cornea. Rabbits were dark-adapted for 1 hour before the measurements were recorded. The study eye was held open with a Barraquer-type wire speculum, and the fellow eye was carefully patched to avoid all stimulation. For recording, we used a photic stimulator (SLS 4100), a biophysical amplifier (AVM-10), and an averager (DAT-1100; Nihon-Kohden, Tokyo, Japan). Time constants were set at 2 seconds for ERG a and b waves, and at 0.003 seconds for oscillatory potentials. Dark-adapted ERGs were recorded with a light stimulus set at 5,000 lux of corneal illuminance (stimulus duration, 0.003 seconds), and recordings were made from the suction-cup type corneal electrode (Kyoto Contact Lens Co., Kyoto, Japan) by averaging 10 responses to the light stimulus at 0.1 Hz. An inactive needle electrode was placed in the eyelid subcutaneously.

Scanning Electron Microscopic Examination

After ERG examination, all rabbits were killed. The 12 o'clock position was marked with a suture placed at the limbus. The enucleated eyes were immediately cut open at the pars plana and placed in 0.1 M phosphate buffer (pH 7.4) containing 2.5% glutaraldehyde and 0.25 M sucrose. After 24 hours fixation, the lens was carefully separated from the posterior segment of the globe. The posterior segment of the globe then was oriented to the anteroposterior axis. After rinsing the buffer, specimens for scanning electron microscopy were fixed in 1% osmium tetroxide for 60 minutes and dehydrated through a graded ethanol series, sputter-coated in palladium platinum, and photographed using a Hitachi S-4100 microscope (Hitachi, Tokyo, Japan).

Results

Indirect ophthalmoscopy and slit-lamp observation indicated that the vitreous was mildly hazy with few infiltrating cells 1 day postinjection with plasmin. These vitreous findings decreased 3 days after injection with plasmin. In eyes that received plasmin and SF_6, the vitreous findings were more apparent. Sulfur hexafluoride resorbed in all eyes 7 days postinjection. Reactions in the vitreous in the eyes that received BSS alone were minimal.

In eyes that received plasmin and SF_6 injection (group 1), scanning electron micrographs showed a smooth retinal surface, consistent with a bare internal

Fig. 1. Scanning electron micrograph of eye with both plasmin and SF_6 injection (group 1). The retinal surface is smooth, which is consistent with a bare internal limiting membrane.

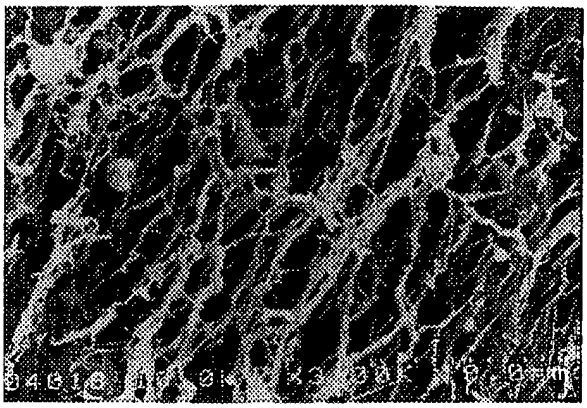

Fig. 3. Scanning electron micrograph of eye with plasmin injection alone (group 3). The retinal surface appears to be covered with sparse collagen fibers.

limiting lamina (Figure 1). These findings were found in both the superior and inferior retina. The vitreous remained attached to the retina at the vitreous base. The retinal surface in eyes injected with plasmin alone (group 2), SF_6 alone (group 3), or BSS appeared to be covered with sparse collagen fibers (Figures 2 and 3).

During electrophysiologic examination 7 days after intervention, the a- and b-wave amplitudes did not differ among eyes that received an injection of plasmin and SF_6, plasmin alone, SF_6 alone, or BSS.

Discussion

Verstraeten and associates[9] demonstrated that injection of plasmin in the rabbit vitreous in combination with a core vitrectomy produced PVD without mechanical peeling of the posterior vitreous cortex from the retinal surface. Vitreous injection of plasmin caused a transient decrease in the ERG b-wave amplitude with excellent recovery. Those authors speculated that the decrease in the ERG amplitude resulted from the high osmolarity of the plasmin solution. Their histologic observations revealed no evidence of retinal damage. In the current study, we injected plasmin combined with SF_6, one of the most popular gas vitreous substitutes to induce PVD. Fineberg and associates[10] reported that no ERG, histologic, or ultrastructural abnormalities in the retina were found in the owl monkey when the vitreous was injected with SF_6.

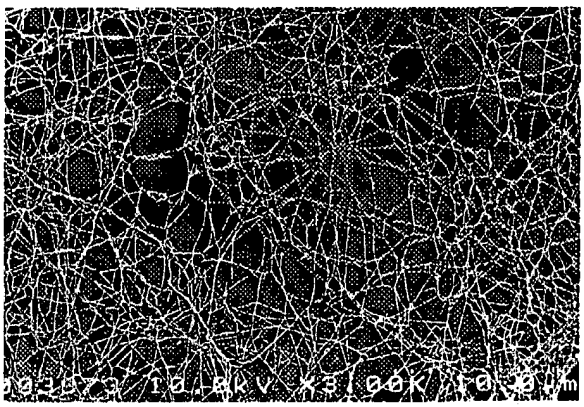

Fig. 2. Scanning electron micrograph of eye with plasmin injection alone (group 2). The retinal surface appears to be covered with sparse collagen fibers.

Plasmin is known to have proteolytic activity against laminin and fibronectin,[11–13] which are components of the internal limiting lamina and thought to bridge and bind vitreous collagen fibers between the posterior vitreous cortex and the internal limiting lamina. Thus, plasmin should exert its activity on the vitreoretinal interface. However, based on the study of Verstraeten and associates,[9] an injection of plasmin alone did not produce PVD. Another intervention, such as a core vitrectomy or SF_6 injection, seems necessary to cause PVD. With core vitrectomy, mechanical oscillation, which is produced by cutting and aspirating the vitreous, might induce separation of vitreous fibers from the internal limiting lamina. Lincoff and colleagues[14] reported that intravitreal injection of the gas induced a loss of hyaluronan and aggregation of vitreous collagen fibers. Biochemical changes such as these in the vitreous might play a role in inducing separation of vitreous fibers from the internal limiting lamina by weakening the adhesion between the internal limiting lamina and vitreous when treated with plasmin.

Our study demonstrated that a vitreous injection of plasmin combined with SF_6 could induce PVD without vitrectomy in rabbit eyes and that a vitreous injection of plasmin alone or SF_6 alone could not induce PVD. A phase II clinical trial of plasmin isolated from the patient's own serum for use during vitrectomy is

currently being organized in the United States.[15] Although the current study has limitations because we used an animal model, if intravitreous gas injection is substituted for vitrectomy, the technique would be simpler, cheaper, and less invasive. A clinical trial is necessary to confirm the efficacy of this technique.

Key words: plasmin, posterior vitreous detachment, sulfur hexafluoride.

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Ophthalmology
Vol-107
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" A New Approach to Stage-3 Macular Holes"
Trese, M.T.; et al
Pages:1607-1611
Year-2000

Seminars in Ophthalmology
Vol-15
Issue-1
" Pharmacological Vitrectomy"
Tanaka, M.; Qui, H.
Pages:51-61
Year-2000

For **Matthew F. DeSanto, AU 3763, 305-3292, US serial NOT GIVEN.**

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Pharmacological Vitrectomy

Minoru Tanaka and Hui Qui

Pharmacological vitrectomy refers to the use of enzymes in an effort to liquefy vitreous and to weaken the adhesion of vitreous cortex to the internal limiting membrane during or before performing vitreous surgery. It is well known that the vitreoretinal interface plays important roles in developing many blinding diseases. To make the vitreous surgery easier for better outcome or to avoid vitrectomy, plasmin, dispase, and chondroinase have been used to promote the disinsersion of vitreous cortex to the internal limiting membrane, a basement membrane of Müller cells.

On the other hand, hyaluronidase has been used clinically to facilitate the clearance of vitreous hemorrhage liquefying vitreous body and developing posterior vitreous detachment. This article reviews enzymes as an intraoperative adjunctive agent in vitrectomy.

ACCORDING to Sebag,[1] vitreous is an extra cellular matrix that fills the center of the eye with a clear viscoelastic tissue that maintains clarity and protects against the untoward effects of eye, head, and body movement. Vitreous is composed of 98% of water when in a gel condition. Its important macromolecules are collagen and hyaluronan, organizing a three dimensional structure[2] with a number of proteoglycans, glycoproteins, and other molecules. It has been thought that these macromolecules play an important role that is responsible for the adherence of the vitreous cortext to internal limiting membrane. Vitreo-retinal adhesion (or interface) consists of the internal limiting membrane and the posterior vitreous cortex. The internal limiting membrane is 3 layers of Müller cells, which consists of lamina rara interna, the lamina densa and the lamina rara externa. The interface has been maintained by laminin, fibronectin, and glycoconjugates functioning as a molecular glue.

The ultimate goal of surgery on the vitreous body is to relieve vitreo-retinal traction or adhesion and facilitate reattachment of the retina. The difficulty in surgery on the vitreous body depends on the grade of adhesion between the vitreous body and retina and the presence or absence of posterior vitreous detachment.[1] In particular, diseases such as proliferative diabetic retinopathy,[3] macular hole,[4,5] and proliferative vitreo-retinopathy are associated with the pathology of the vitreo-retinal interface. However, retinal break forms during surgery in a high population of young patients and patients with marked adhesion, and the incidence of complication is high.

Recent studies have been developed for promoting liquefaction of the vitreous body and for relieving adhesion of the vitreo-retinal interface by using some enzymes (Table 1).[6] This pharmacological manipulation of the interface with enzymes is called pharmacological vitrectomy.

This article reviews recent studies on the enzyme assisted vitrectomy. In addition, our recent studies on enzyme assisted vitrectomy with hyaluronidase are reported.

ENZYMES CURRENTLY USED

Plasmin

Plasmin is a nonspecific protease that is isolated from the patient's own serum. Plasmin lyses laminin and fibronectin and also activates several matrix metalloproteinases (MMP) and facilitates PVD without causing any ill effects.[7] This protease has been used clinically for the treatment of macular holes in young patients and diabetic macular edema. According to Williams,[8,9] autologous plasminogen is isolated from the patient's plasma with a lysin affinity column and then converted to plasmin with streptokinase. Plasmin is injected into the patient's vitreous cavity 15 minutes before vitrectomy. Core vitrectomy and separation of posterior vitreous cortex from the retina is performed. In younger patients, plasmin acts to facilitate the detachment of the posterior vitreous from the retina. Remarkable results are presented in younger patients with traumatic macular holes. In addition, they introduce successful results in treating diabetic macular edema, in which plasmin has been injected without vitrectomy and successfully induced PVD

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Table 1. Pharmacologic Vitreolysis

Type of Vitreolysis	Treatment
Enzymatic	
Nonspecific	Plasmin
	Dispase
Substrate-specific	Chondroitinase
	Hyaluronidase
	Collagenase
Nonenzymatic	

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with visual improvement. A phase II and III clinical trials of this agent is organized in the United States.

Dispase

Dispase is a neutral 35.9 kd protease obtained from Bacillus Prolymyxa, which cleaves the basal lamina in various tissue including skin, testis, and retinal pigment epithelium.[10] This enzyme acts on type IV collagen and fibronectin; however, it does not act on type V and VI collagens and laminin. Tezel et al[11] used this enzyme to study whether or not dispase selectively cleave the attachment of the posterior vitreous to the inner limiting membrane with enucleated porcine and human eyes. This agent was injected into the vitreous cavity of enucleated human eyes (5 U/mL) and incubated at 37°C for 15 to 120 minutes. An extent of PVD was graded, and light electron and scanning electron microscopic observation were performed. As a result, they observed almost complete PVD in human cadaver eyes after incubation with 5 U/mL dispase for 15 minutes. Dispase cleaved the attachment of the posterior vitreous to the inner limiting membrane with minimal damage to the retina. The authors concluded that dispase might be useful in removing cortical vitreous during vitreous surgery.[11] The action of this enzyme is only on postmortem eyes, and no evidence of vitreous liquefaction were shown.

Chondroitinase

A substrate specific chondrotinase has been thought to be effective in inducing disinsertion of the vitreous body. This enzyme lyses chondroitin sulfate, which might participate in vitreo-retinal adhesion. Hageman et al[12] conducted the studies to determine the feasibility of using this enzyme to evoke disinsertion of the vitreous body. They observed that the intravitreal injection of this enzyme elicits complete disinsertion of the vitreous body from the retina, including the vitreous base within 5 to 15 minutes. The internal limiting membrane remained intact and no adverse effects were observed. They also reported that chondroitinase has been used to detach epiretinal membranes from the retina, providing evidence that this proteoglycan participates in pathological adhesion to internal limiting membrane. They concluded that the use of chondroitinase will simplify vitrectomy, especially in the treatment of epiretinal membrane and macular holes.

On the other hand, chondroitinase ABC was also used to degradate the proteoglycan by intravitreal injection.[13] Morimoto et al[13] studied appropriate concentration and reaction time of intravitreal injection of the enzyme, in which degradation reached a plateau at 10 minutes with no adverse effect. From these results, chondroitinase ABC may be an effective and safe method to create retinal detachment when macular relocation is needed.[13]

Hyaluronidase

Hyaluronidase, the substrate specific enzyme, has also been tried to liquefy vitreous body. This specific enzyme cleaves hyaluronan into disaccharide components by splitting the bond between the glucosamin and glucuronic acid. Although, in the past, the use of this enzyme resulted in extensive retinal necrosis, recent studies using highly purified at lower doses of hyaluronidase documented the efficacy of vitreous liquefaction of the vitreous body.[14,15]

Harooni et al[15] showed that intravitreal injection of hyaluronidase in doses of 10 IU or higher induced posterior vitreous detachment in rabbits and concluded that injection of hyaluronidase could be considered as an alternative or adjunct to conventional mechanical vitrectomy. In addition, there is a report in which PVD is induced successfully by intravitreal injection of hyaluronidase and sulfur hexafluoride.[16] In addition, hyaluronidase has been used for nonclearing vitreous hemorrhage.[17,18]

EFFICACY OF HYALURONIDASE TO LIQUEFY VITREOUS AND TO FACILITATE THE CLEARANCE OF VITREOUS HEMORRHAGE

To facilitate liquefaction of the vitreous body, the safe and effective concentration and reaction time of hyaluronidase were evaluated.[17] Under the con-

ditions evaluated, promotion of vitreous hemorrhage absorption was examined in an animal model in which autologous whole blood was injected into the vitreous cavity.

Experiment on Liquefaction of the Vitreous Body

Hyaluronidase solution (0.1 mL) of 40, 20, 10, and 5 U, respectively, was injected into the vitreous cavity of pigmented rabbits. In addition to routine clinical observations, electrophysiological, funduscopic, and histopathological studies were performed. The clinical examinations revealed that 15 and 30 minutes after injection of enzyme solution, there were no marked findings in the anterior segment or vitreous cavity in any group. Electroretinogram (ERG) showed a slight reduction of amplitude in the group in which 40 U of hyaluronidase was injected and the reaction time was 30 minutes, as shown in Figure 1. However, there were no marked differences among the groups. Clinical examination did not clarify the presence or absence of PVD or vitreous liquefaction in vivo. In each group, neither clinical examination nor ERG showed any changes 15 minutes after injection.

In the group with digestion 30 minutes after injection of 40 U, retinal edema was observed in an area where it appeared that the retina had been extensively exposed to enzyme solution. Furthermore, local retinal detachment was detected (Fig 2). Approximately 1 mL of fluorescein Na solution was dropped on the vitreous body. Thereafter, eye cups were washed in physiological saline, and the grade of liquefaction was investigated. Liquefaction at the center of the vitreous cavity, which may have been digested by enzyme solution based on the site colored with fluorescein Na solution, was observed (Fig 3A). In control eyes, the residual vitreous body was colored (Fig 3B). Light microscopy revealed a large number of vacuoles at the retinal edema site. Outer segments were disordered (Fig 4). Electron microscopy revealed vacuolation in the outer layer and disorder in the sequence of outer segments (Fig 5A). In the internal layer, there were no abnormalities in Müller's cells or the internal limiting membrane. A large number of vacuoles were observed in the nerve fiber layer (Fig 5B). On hanging test, the length of the residual undigested vitreous body in eyes receiving 40 U of hyaluronidase was markedly shorter than that in control eyes (Fig 6B), suggesting severe digestion (Fig 6A).

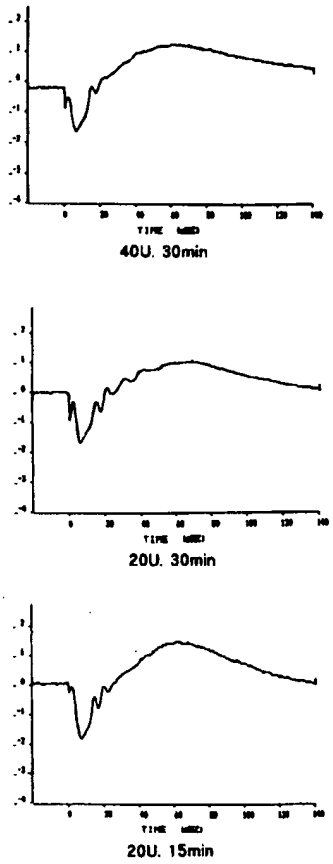

Fig 1. Electroretinograms (ERGS) after injection of hyaluronidase. The upper row shows ERG 30 minutes after injection of 40 U of hyaluronidase. The middle row shows ERG 30 minutes after injection of 20 U of hyaluronidase. The lower row shows ERG 15 minutes after injection of 20 U of hyaluronidase. A slight reduction of amplitude was noted 30 minutes after injection of 40 U of hyaluronidase (upper row).

Among eyes receiving 20 U of hyaluronidase, there were no abnormalities or liquefaction detected in the group with digestion 15 minutes after injection. Under a stereoscopic microscope, extremely local retinal edema was observed in the group in which 20 U of hyaluronidase was injected with a reaction time of 30 minutes, as noted in the group in which 40 U of hyaluronidase was injected with a reaction time of 30 minutes. However, retinal detachment was not detected. In eyes receiving 20 U of hyaluronidase, light microscopy did not show any vitreous body on pentaacetic acid staining (Fig 7A), suggesting sufficient liquefaction in comparison to that in control eyes (Fig 7B). Light microscopy revealed slight vacuolation in the in-

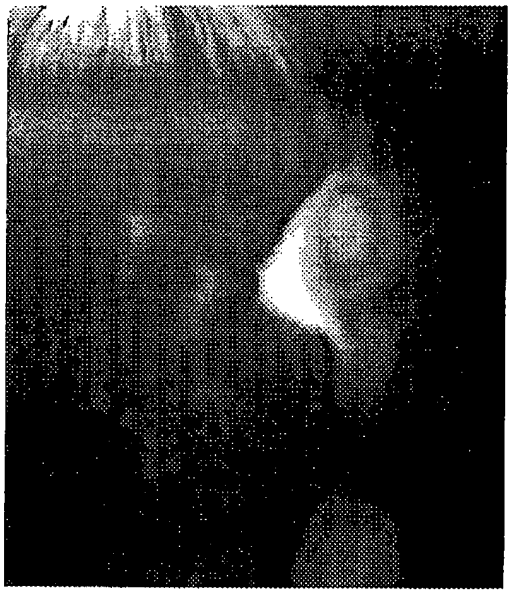

Fig 2. A stereoscopic microscopic photograph 30 minutes after injection of 40 U of hyaluronidase. Partial retinal edema and retinal detachment were observed.

ternal layer in the enzyme injected groups (Fig 7A). Electron microscopy showed slight vacuolar degeneration in the outer retinal layer. However, there were no abnormal findings detected in the inner retinal layer. On hanging test of the undigested vitreous body, the grade of digestion was lower than that in eyes receiving 40 U of hyaluronidase. However, eyes receiving 20 U of hyaluronidase showed sufficient digestion (Fig 8) compared with that in control eyes (Fig 6A).

Experiment on Promotion of Vitreous Hemorrhage Absorption

The results of the liquefaction experiment proved that the safe and effective concentration required for liquefaction of the rabbit vitreous body was 20 U and that a reaction time of 30 minutes was useful. Autologous blood was collected through the ear vein. In group 1, autologous blood (0.1 mL) was injected into the unilateral eye. In group 2, autologous blood and 20 U of enzymes (0.1 mL) were injected into the unilateral eye.

Balanced Salt Solution (BSS) (0.1 mL) was injected into the fellow eye as a control agent. Twenty-four hours and 1 week after injection, the eyes were used for the following examinations.

Immediately after injection, slit lamp microscopy and funduscopy were performed. Twenty-four hours and 1 week after injection, similar clinical examinations were performed, and eyes were extirpated to prepare eye cups. Light microscopy and electron microscopy were performed. Funduscopy was performed daily until 1 week after injection to investigate the process of absorption.

As a result, in eyes in which 0.1 mL of autologous blood alone was injected and eyes in which 0.1 mL of autologous blood and 0.1 mL of hyaluronidase (20 U) were injected, the transparency of the fundus was relatively reduced im-

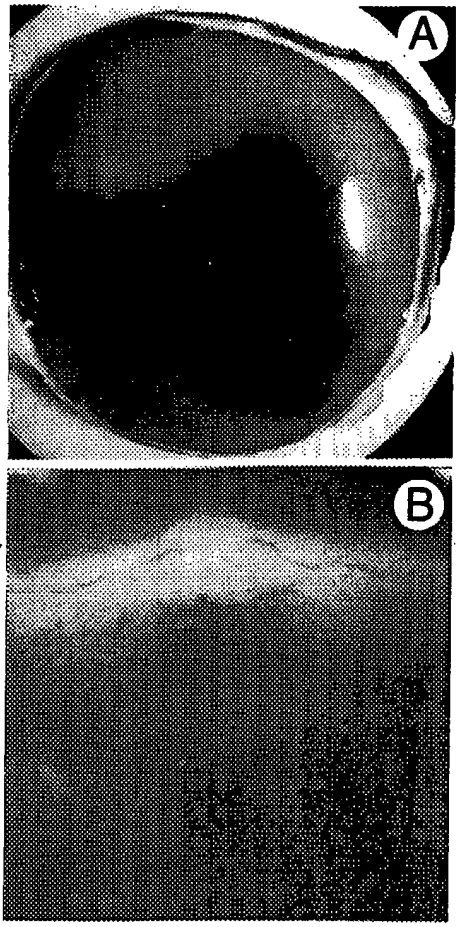

Fig 3. (A) A photograph after the eye cup was prepared 30 minutes after injection of 40 U of hyaluronidase and stained with fluorescein Na, then washed. The center on which this enzyme acted showed liquefaction. The vitreous body disappeared, and was replaced by water. (B) Control group in which enzyme was not injected. The entire eye cup was stained. There was no digestion.

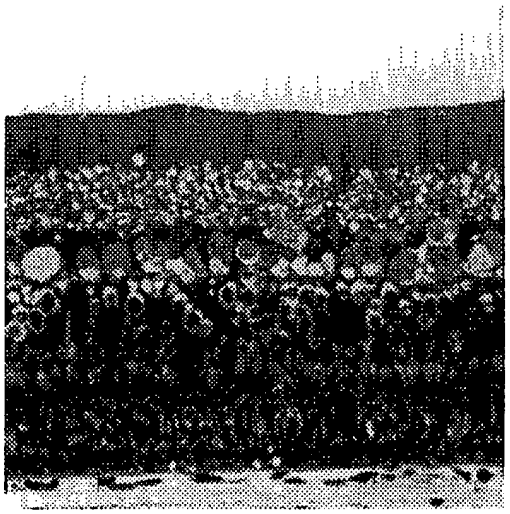

Fig 4. Light microscopy of a site in which stereoscopic microscopy showed retinal edema 30 minutes after injection of 40 units of hyaluronidase. Vacuoles were observed in the retinal layers. The sequence of outer segments was disordered (toluidine blue staining 100 ×).

mediately after injection. Photographs on funduscopy are shown in Figure 9. Twenty-four hours after injection, there was no significant difference in the grade of hemorrhage absorption between the group receiving autologous blood alone and the group receiving autologous blood and enzyme solution. As shown in Figure 10B, blood components adhered to undigested vitreous fibers and remained in both groups. There was no efficacy 24 hours after injection. Seven days after injection, there were no changes in eyes in which autologous blood alone had been injected. The transparency of the fundus was poor. However, in eyes in which enzyme solution was simultaneously injected, the transparency of the fundus became almost normal. Stereoscopic microscopy of eye cups showed complete absorption of hemorrhage except for the residual coagulation mass below (Fig 10A).

Histopathological examinations 7 days after injection revealed that a large volume of vitreous fibers on the retinal surface in the group in which autologous blood alone was injected. A large number of blood cell components adhered to these vitreous fibers in a complex way (Fig 11A). However, in the group in which autologous blood and hyaluronidase were injected, the residual vitreous body was fragmented, and the volume was extremely small. Therefore, the volume of blood cell

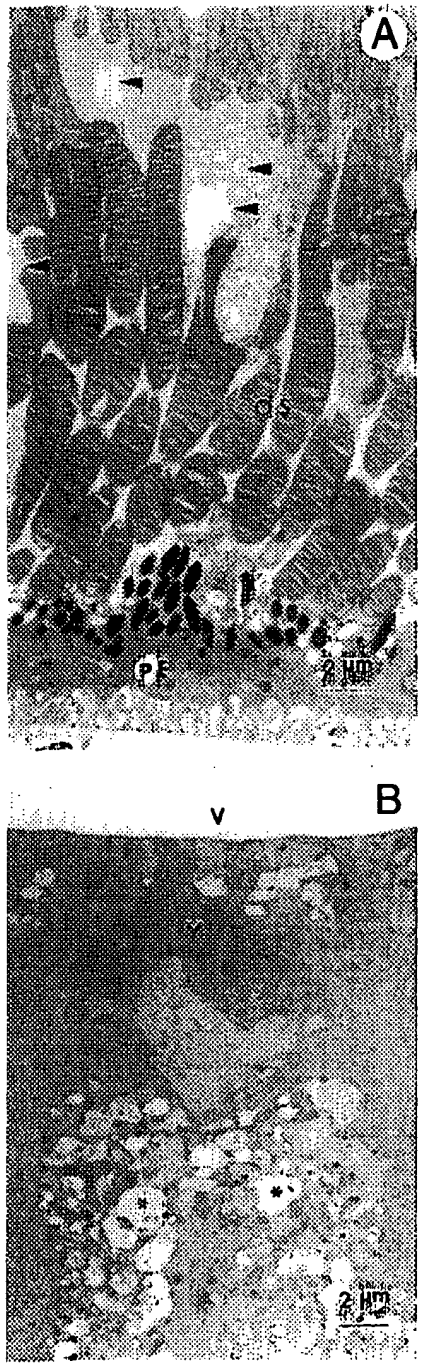

Fig 5. (A) Electron microscopy 30 minutes after injection of 40 units of hyaluronidase. Vacuolar degeneration (arrow heads) was observed in the outer segment layer. The sequence of outer segments was disordered. OS, outer segment; PE, pigment epithelium (bar: 2 μm). (B) In the internal retinal layer. Müller cells were normal. However, a large number of vacuoles were observed in the nerve fiber layer (asterisk). V, vitreous; M, Müller's cell (bar: 2 μm).

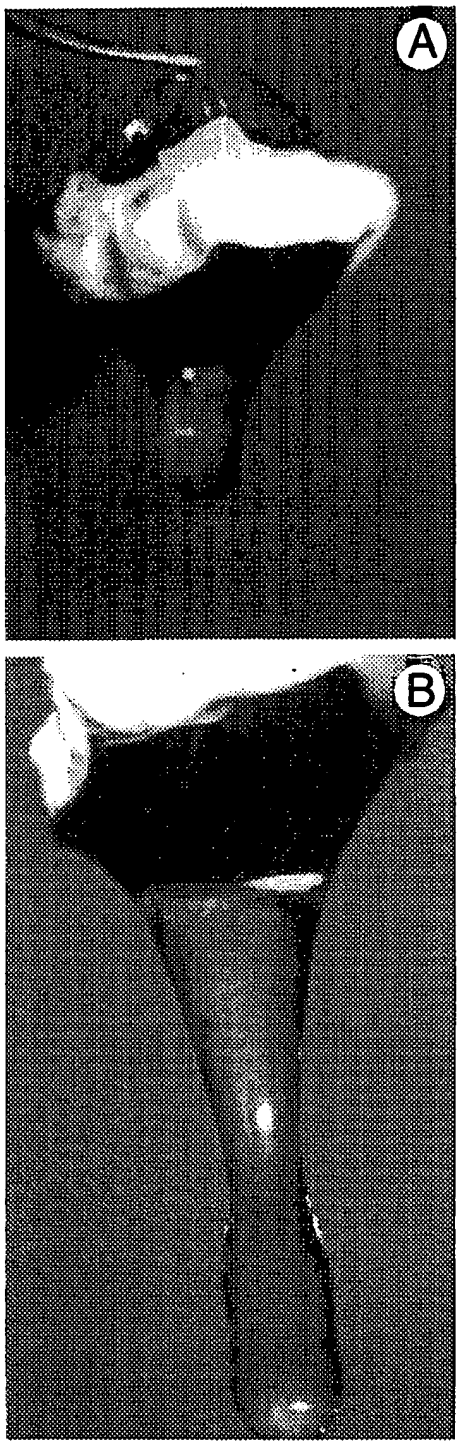

Fig 6. Experiments of liquefaction 30 minutes after injection of 40 U of hyaluronidase. In eyes in which hyaluronidase was injected, most vitreous bodies (A) showed liquefaction (B) compared with those in control eyes.

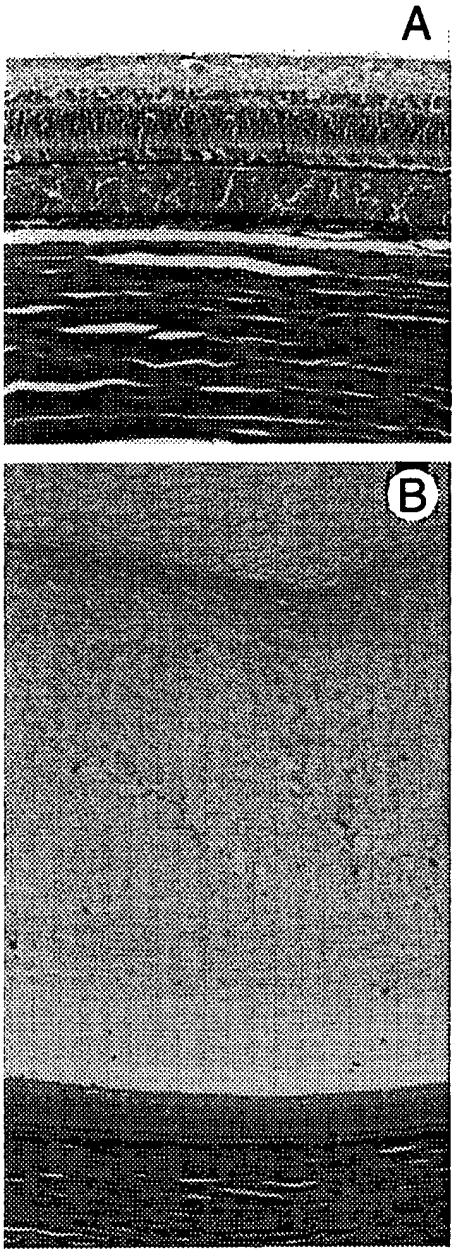

Fig 7. (A) Light microscopy 30 minutes after injection of 20 U of hyaluronidase. In eyes in which hyaluronidase was injected, there were no vitreous bodies observed. (B) However, in control eyes, a large volume of vitreous bodies were observed adjacent to the internal limiting membrane. (Pentaacetic acid staining 20 ×).

Fig 8. Vitreous body liquefaction 30 minutes after injection of 20 U of hyaluronidase. Sufficient liquefaction was observed compared to that in control eyes as shown in Fig 6B.

components adhering to vitreous fibers was also extremely small (Fig 11B). There were no abnormal retinal findings observed in either group. On toluidine blue staining, a large number of hemosiderin particles were detected on the internal limiting membrane (Fig 12A). When this site was examined under an electron microscope, hemosiderin particles were adhered and sequenced on the internal limiting membrane, and partial thinning of the internal limiting membrane was observed (Fig 12B). Further examination revealed a small volume of fine vitreous fibers, but the structure was fragmented, whereas the volume was decreased in the group in which enzyme solution was injected (Fig 13A). However, a sufficient volume of fine vitreous fibers remained in the group without enzyme solution, while the fibrous structure was sustained (Fig 13B). However, in both groups, the internal limiting membrane remained. In this area, digestion of the membrane was not observed, and presence of PVD was not clear (Figs 13A and B). In some areas, hemosiderin particles may have pushed down the internal limiting membrane, destroying the vitreo-retinal interface structure (Fig 13C).

Harooni et al[15] reported that injection of 10 U or more of hyaluronidase facilitated vitreous body liquefaction and that 20 U or less of hyaluronidase did not cause any side effects histologically or functionally. Furthermore, they indicated that a prolonged period, 5 weeks or more, was required to induce PVD by hyaluronidase. In this study, 10 U of hyaluronidase facilitated vitreous body liquefaction, but the finding was insufficient. Twenty units or more of hyaluronidase facilitated sufficient vitreous body liquefaction. However, exposure to 40 U of hyaluronidase for 30 minutes induced abnormal findings on ERG and histopathological examination. Therefore, clinical application may not be appropriate. Exposure to 20 U of hyaluronidase for 30 minutes may be effective during surgery on the vitreous body or immediately before surgery in patients who do not clinically show vitreous body liquefaction. In other words, it is suggested that hyaluronidase may be a useful intraoperative device. In addition, Harooni et al reported that enzyme injection for 5 weeks or more was required to prepare PVD. However, in our experiment, the internal limiting membrane was intact 1 week after injection of autologous blood and hyaluronidase, and PVD was not clear. Briefly, hyaluronidase did not digest the internal limiting

Fig 9. Funduscopic finding after 0.1 mL of autologous blood was injected into the vitreous body cavity of colored rabbits. Neither the optic nerve nor medullated nerves were observed because of blood in the vitreous body.

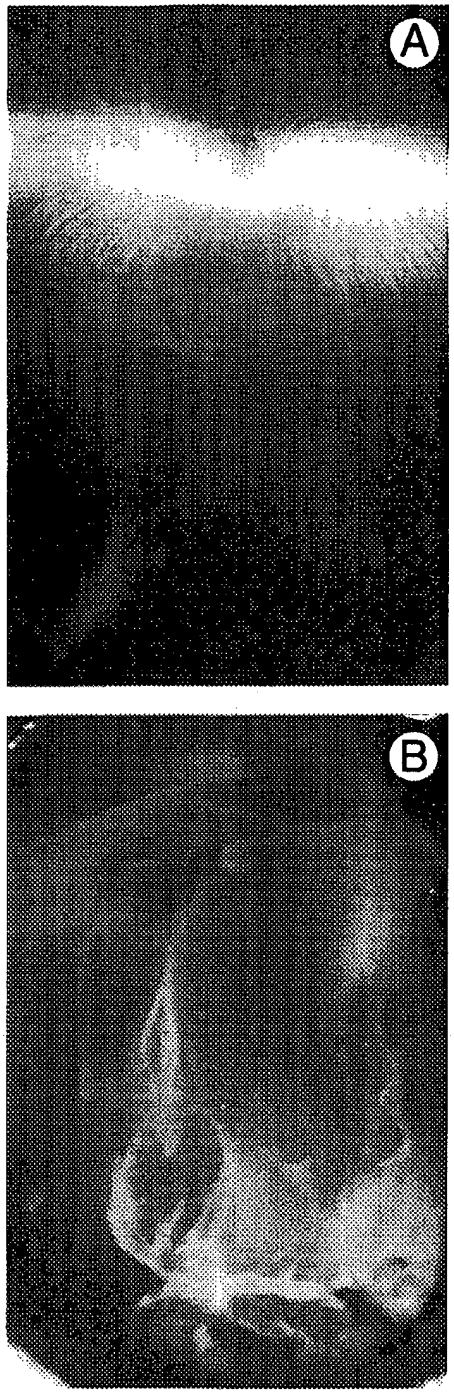

Fig 10. (A) In eyes in which autologous blood and 20 U of hyaluronidase were injected, vitreous body cavity blood was absorbed except for blood coagulation in the inferior direction. (B) However, in eyes in which autologous blood alone was injected, blood components adhered to vitreous fibers, and remained without being absorbed.

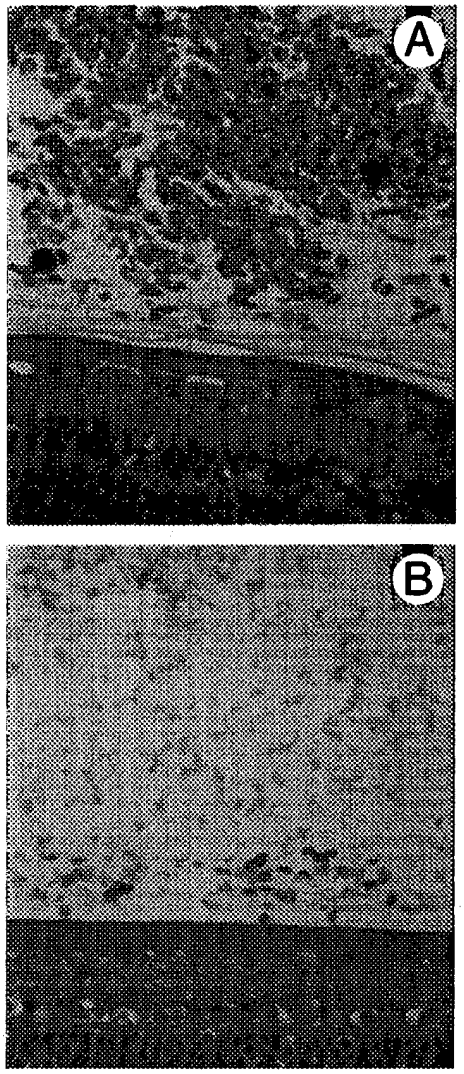

Fig 11. Light microscopy 7 days after injection. In eyes in which autologous blood alone was injected, abundant vitreous fibers remained. (A) Therefore, a large number of blood cell components adhering to these fibers were observed. However, in the group in which autologous blood and hyaluronidase were injected, vitreous fibers were fragmented. (B) Furthermore, the number of blood cell components was small. (Pentaacetic staining 200 ×).

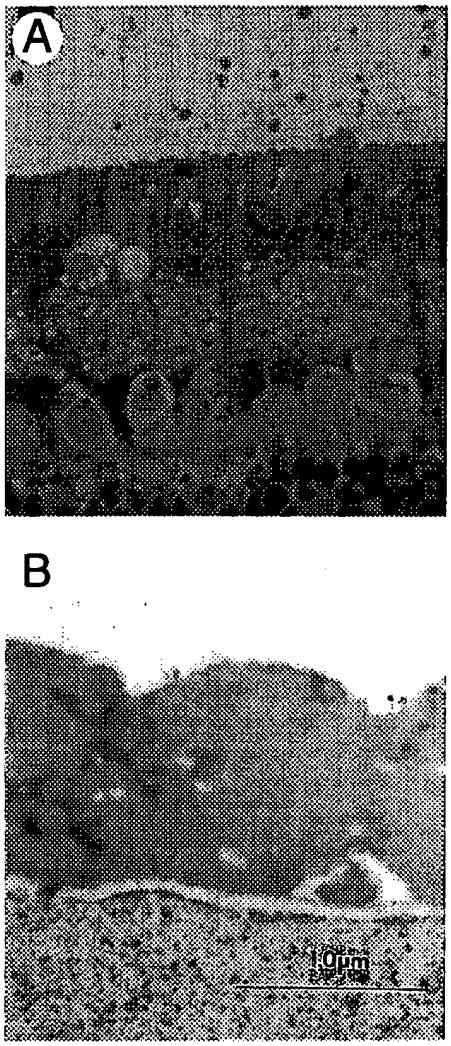

Fig 12. Light microscopy 7 days after injection. (A) After blood components were absorbed, a large number of hemosiderin granules were partially observed on the internal limiting membrane. (Toluidine blue staining 200 ×). (B) When this site was examined under an electron microscope, the internal limiting membrane remained. However, partial thinning was noted in some areas (bar: 1 μm).

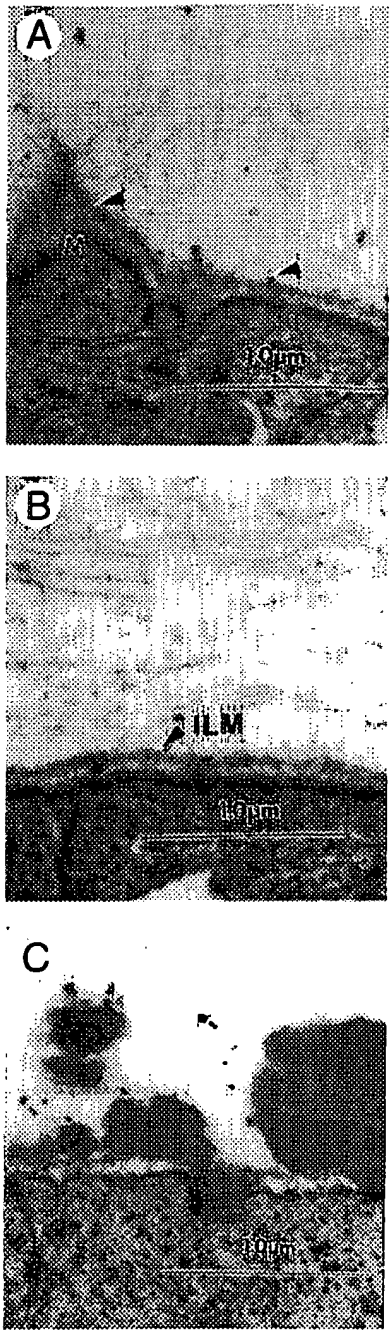

Fig 13. Electron microscopy of the periphery of the internal limiting membrane. (A) In the group in which autologous blood and hyaluronidase were injected, (B) vitreous fibers were fragmented compared to those in the group in which this enzyme was not injected. However, in both groups, the internal limiting membrane remained. PVD was not observed. (C) In some areas, hemosiderin particles destroyed the structure of the internal limiting membrane, invading Müller's cells (bar: 1 μm).

membrane. In the future, the action of hyaluronidase on the vitreo-retinal interface should be further investigated.

This study investigated the possibility of vitreous body liquefaction and promotion of absorption in patients with vitreous hemorrhage only for which simple vitrectomy alone may be indicated. Karagozian et al[18] reported that hyaluronidase was

effective in patients with vitreous hemorrhage without spontaneous absorption. Recent studies with Vitrase showed that this enzyme improved the rate of hemorrhage clearance by augmenting the cellular responses,[19] and that the highest dose was associated with the highest incidence of clearing of vitreous hemorrhage.[20] The results of our experiment also showed that injection of 20 U of hyaluronidase promoted absorption. Therefore, in patients with vitreous hemorrhage only for which simple vitrectomy should be indicated, for example, in patients with diabetic retinopathy, the use of this enzyme may accelerate hemorrhage absorption, facilitating appropriate photocoagulation treatment without vitrectomy.[21] Furthermore, complications related to surgery on the vitreous body may be prevented. In many patients with vitreous hemorrhage related to retinal central (branch) venous occlusion, simple resection alone is required. Therefore, hyaluronidase can also be applied in patients with this disease. Furthermore, this enzyme may be indicated for patients in whom surgery is difficult because of systemic disorders. In addition, considering that PVD develops 5 weeks after injection,[14] this enzyme may inhibit various lesions on the vitreoretinal interface that may occur after hemorrhage absorption.

COLLAGENASE

Clostridiopeptidase A (bacterial collagenase), in highly purified form, was used to facilitate removal of epiretinal membranes by partial digestion of epiretinal collagenous fibrous tissues before mechanical surgery. Intravitreal injection showed no adverse effects.[22] Because no toxicity was seen when collagenase was injected before vitrectomy in animal study, pilot human study was performed.[23]

Moorhead et al[22-23] reported that 6 patients underwent pars plana vitrectomy. In each case, removal of some dense fibrous tissues was performed by standard mechanical methods. When removal of additional fibrous tissue was dangerous, collagenase was injected intravitreally.

Twelve units of collagenase were used in the first 3 cases to ensure safety. A higher dosage (24 U) was used in the last 3 cases. Incubation time was 15 minutes. Six patients consisted of 2 cases of premature retinopathy, 3 cases of diabetic retinopathy, and 1 case of proliferative vitreoretinopathy. As a result, they observed dot-like petechial hemorrhages on the surface of the fibrous tissue after 15 minutes of incubation. No retinal tears, hemorhages, or lens changes were observed after operation. They concluded that the use of this enzyme may facilitate removal of fibroproliferative tissue in certain difficult vitrectomy cases and that further investigation is necessary to establish the optimum dosage to enhance membrane removal without causing damage to the retina and crystalline lens. Our previous study showed that collagenase relieves intraocular proliferative changes, but promotes digestion of retinal vessels, causing hemorrhages.[24]

CONCLUSIONS

The purpose for using enzymes in treating vitreoretinal disease is to liquefy vitreous body and to weaken the adherence of posterior vitreous cortex to the internal limiting membrane leading to disinsertion away from retina. From these concepts, this new method will facilitate and enhance many surgical procedures currently used. If these methods develop, vitreous surgery will be replaced in managing certain kinds of diseases by noninvasive therapy, such as pharmacological vitreolysis in the future.

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Jacob, Rebecca (ASRC)

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From: STIC-ILL
Sent: Wednesday, December 04, 2002 10:58 AM
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Subject: FW: Articles

-----Original Message-----
From: Solomon, Terrance
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The following articles **were not** available full-text using STIC resources:

Ophthalmologe
Vol-98
Issue-12
" Using Enzymes in the Posterior Eye Segments. Current Status and Future Possibilities"
Hesse, L.
Pages:1176-1180
Year-2001

American Journal of Ophthalmology
Vol-133
Issue-1
"Vitreoretinal Morpholgy of Plasmin-treated Human Eyes"
Gandorfer, A.; et al
Pages:156-159
Year-2002

For **Matthew F. DeSanto, AU 3763, 305-3292, US serial NOT GIVEN**

Thanks,

Terry Solomon
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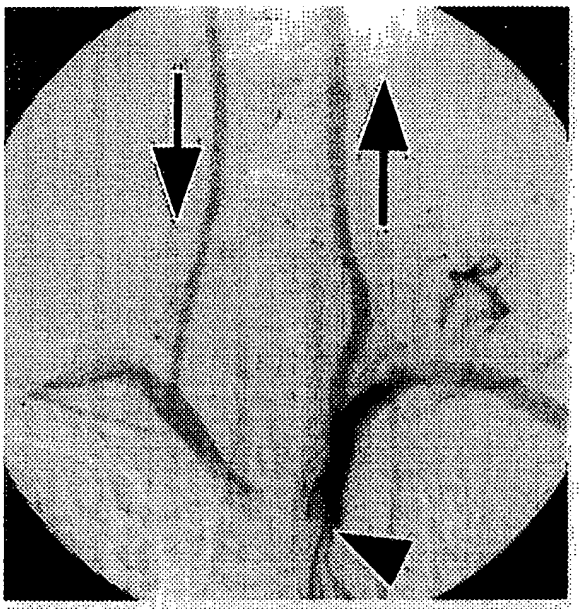

FIGURE 2. Digital subtraction angiography, the catheter (arrowhead) is placed in the left subclavian artery. Injection reveals retrograde filling of the right vertebral and subclavian arteries typical of a subclavian steal phenomenon. (Arrows indicate blood flow directions.)

corresponds to the "Innominate Steal Syndrome" described in 1965 by Blakemore and associates[3] as a proximal counterpart of the subclavian steal syndrome (Figure 2).

Interference with the circulation through the brachiocephalic trunk results in a variety of symptoms reflecting ischemia in both carotid and vertebrobasilar arterial territories: Transient monocular blindness indicates a disease in the carotid artery territory, whereas nausea and vertigo are common for vertebrobasilar dysfunction.

Some information suggesting cerebrovascular disease may also be obtained from the globe: Before dilation of the stenotic vessel, the pathologic OPA of this patient reflects the impaired arterial supply to the right eye and the enhanced blood flow to the left eye respectively. After dilation, the reduction in the inter-eye OPA difference mirrors the normalization in cerebral circulation.

Abnormalities of the ocular pulse amplitude are known to occur not only with ocular diseases. Perkins[4] found that "abnormalities in the ocular pulse . . . have a diagnostic value similar to that of other noninvasive tests" for carotid stenosis, particularly Doppler sonography. He argued that since carotid stenosis is both serious and treatable, it would be worthwhile screening for it during a routine eye examination if this was technically possible. Handled like a conventional contact lens, this recording applanation tonometer might prove a valuable device for identifying hemodynamic lesions in an office setting.

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Vitreoretinal Morphology of Plasmin-Treated Human Eyes

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PURPOSE: To investigate the ultrastructure of the vitreoretinal interface following an intravitreal injection of plasmin.

METHODS: Plasmin (2 U/0.1 ml) was injected into the vitreous cavity of five postmortem human eyes. The five fellow eyes received phosphate-buffered saline and served as controls. After incubation at 37°C for 30 minutes, the globes were placed in fixative and hemisected. Specimens for scanning and transmission electron microscopy were obtained using a corneal trephine.

RESULTS: All plasmin-treated eyes showed complete vitreoretinal separation with sparse collagen fibrils covering the inner limiting membrane. All control eyes showed an attached cortical vitreous. At the vitreous base, there was no cleaving effect. The retinal morphology of plasmin-treated eyes was unchanged.

CONCLUSIONS: Plasmin induces a cleavage between the vitreous cortex and the inner limiting membrane without morphologic alteration of the retina. (Am J Ophthalmol 2002;133:156–159.

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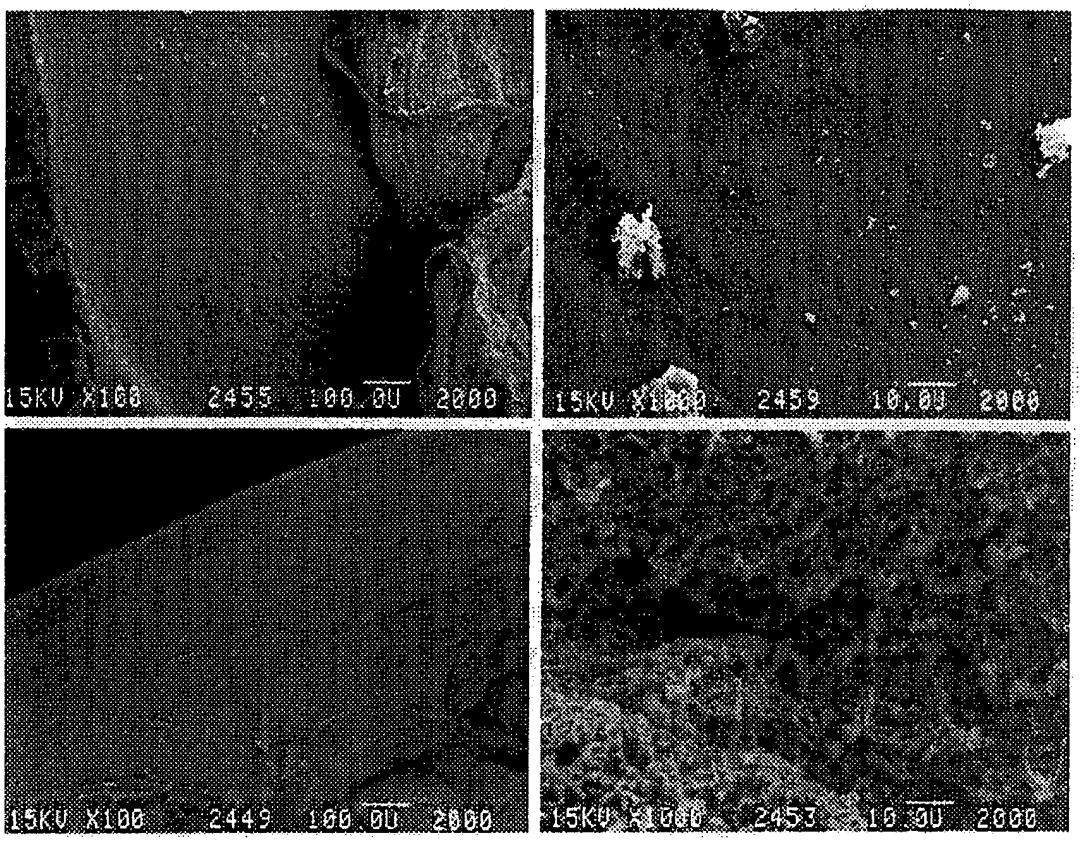

FIGURE 1. Scanning electron microscopy of plasmin-treated eyes and controls. (Top) There is complete posterior vitreous detachment following plasmin treatment. (Bottom) All control eyes show an attached hyaloid with densely packed collagen fibrils.

IT HAS BEEN REPORTED PREVIOUSLY THAT PLASMIN FACILitates vitreoretinal separation.[1,2] Recently, injection of autologous plasmin into the midvitreous cavity followed by lavage and long-acting gas filling and head-down positioning has been proposed as a novel approach to stage 3 macular holes.[3] However, there are no data available concerning the cleaving effect and dosage of plasmin in human eyes. Therefore, we investigated the morphology of the vitreoretinal interface of human postmortem eyes following an intravitreal injection of plasmin.

Ten healthy human globes were obtained from the local eye bank having been removed from five donors within eight hours of postmortem time. The donors' ages ranged from 54 to 76 years. The globes were incubated in a moist chamber at 37°C for 15 minutes. Human plasmin (2U/0.1 ml; EC, Sigma-Aldrich, Germany) was injected into the vitreous cavity of five eyes; five fellow eyes received 0.1 ml of calcium- and magnesium-free phosphate-buffered saline and served as controls. After incubation at 37°C for 30 minutes, four sclerotomies were made at the pars plana to ensure rapid penetration of the fixative. The globes were fixed in a solution of 4% formaldehyde at 4°C for 24 hours, and hemisected. Retinal specimens for scanning and transmission electron microscopy were obtained from the posterior pole, the equator, and the vitreous base using a corneal trephine.

All plasmin-treated eyes showed vitreoretinal separation. Electron microscopy revealed a smooth retinal surface with sparse remnants of collagen fibrils (Figure 1 top). All control eyes showed an attached vitreous. Persistent vitreous attachment was confirmed by electron microscopy. Densely packed collagen fibrils covered the inner limiting membrane (Figure 1 bottom). At the vitreous base, the vitreous cortex was attached to the retina in plasmin-treated eyes and controls. All eyes showed intact retinal structures throughout the specimens (Figure 2).

Recently, we demonstrated that plasmin produced a complete and dose-dependent cleavage at the vitreoretinal interface of porcine postmortem eyes without damaging the retina.[4] The present study confirms the cleaving impact of plasmin in human eyes. However, regarding the key role of plasmin in degrading the extracellular matrix, untowards effects on the retina may bias the advantages of enzymatic vitreoretinal separation.[5] Histologic work up revealed no evidence of retinal damage in any plasmin-treated eye. Even at a high concentration of 2 U of plasmin for 30 minutes, there was no change of the retinal morphology. The ultrastructure of the ILM was especially well preserved.

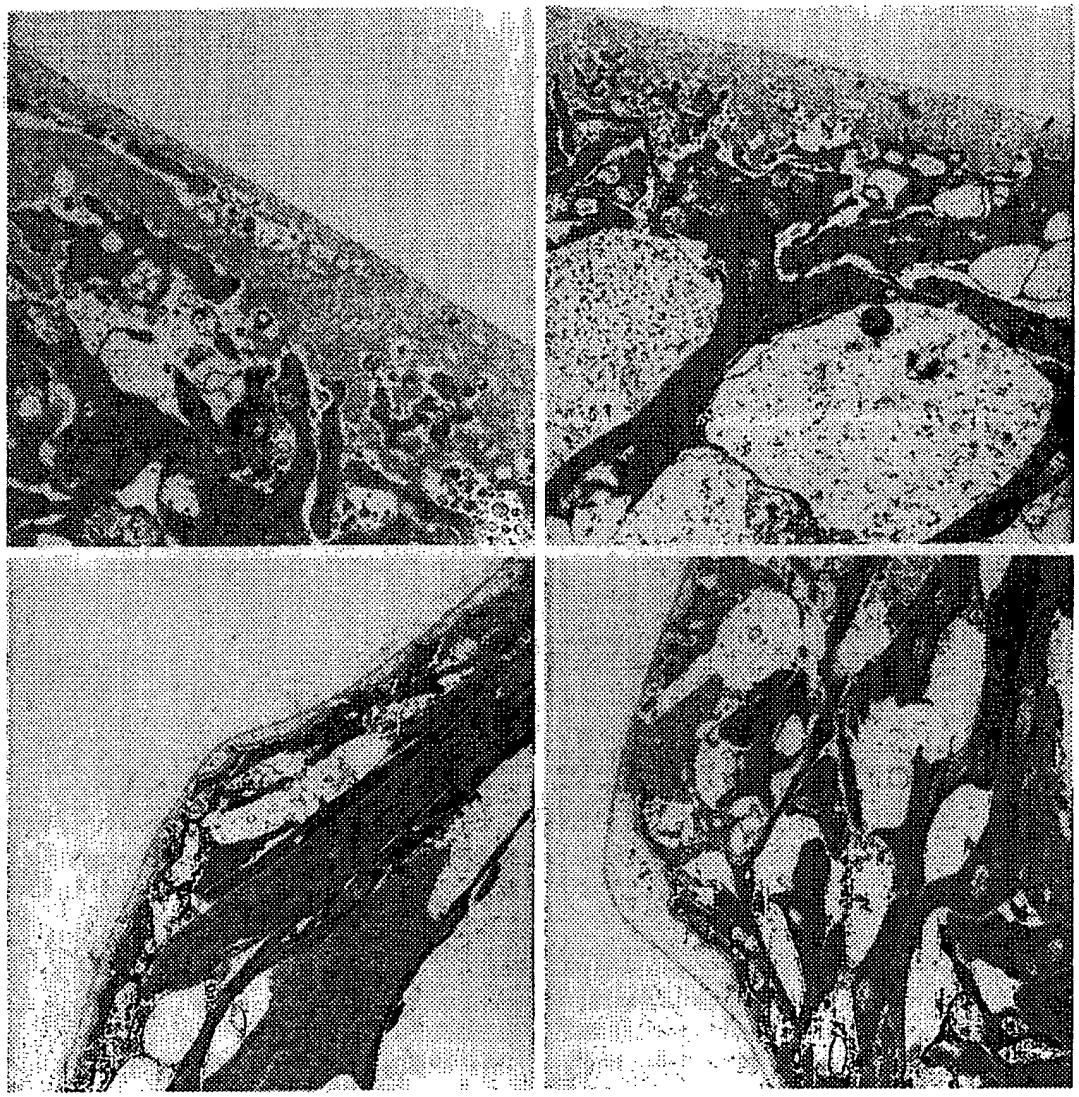

FIGURE 2. Regarding the ultrastructure of the inner retina, transmission electron microscopy shows no difference between plasmin-treated eyes (left) and controls (right). (Top) Macular area (top left 16000×, top right 9500×). (Bottom) Vitreous base (3600×).

Besides the proteolytic activity of plasmin against laminin and fibronectin, side effects of plasmin such as activation of collagenases may have contributed to vitreoretinal separation. In our series, however, the vitreous cortex remained completely attached at the vitreous base in plasmin-treated eyes and controls. This may indicate that plasmin did not cleave the vitreoretinal junction by secondary activation of collagenases.

The presence of complete vitreoretinal separation in all plasmin-treated eyes compared with the attached hyaloid in controls emphasizes the efficacy of plasmin in cleaving the vitreoretinal junction. The unchanged morphology of the retina may demonstrate the safety of plasmin in this experimental setting. It is of note, however, that conclusions regarding the physiology of the retina cannot be drawn from this study.

Further studies are now required to confirm the cleaving effect of plasmin at the vitreoretinal interface of human eyes *in vivo*, and to rule out any untoward effect to the retina or other intraocular structures.

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examination the lining looked irregular and there were small white particles which appeared to be floating away from the sleeve. There was no sign of any irregularities 'downstream' from the tip, either in the irrigation fluid and tubing or in the phacoemulsification handpiece.

We concluded that these particles had originated from the phacoemulsification tip sleeve which had had a manufacturing defect. The inert nature of the retained particle would be in keeping with the presumption that it was a silicone fragment. The sleeve and fluid from the phacoemulsification cassette were sent to the manufacturer for analysis but were lost in transit.

We would recommend the routine inspection of the sleeve to look for any manufacturing abnormalities.

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Sir,

Plasmin-assisted vitrectomy eliminates cortical vitreous remnants

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Plasmin, a non-specific serine protease mediating fibrinolysis, has properties to hydrolize a variety of glycoproteins, including laminin and fibronectin.[1] By degrading the links between these components of the vitreoretinal interface and the inner limiting membrane (ILM), therapeutic posterior vitreous detachment (PVD) has become possible.[2,3] In controlled experiments in postmortem porcine eyes, enzymatic action alone is sufficient to induce PVD.[2] However, there are remnants of cortical vitreous remaining adherent to the ILM depending on the dose and exposure time of plasmin.[2]

Enzymatic vitrectomy is envisaged to augment or even replace conventional vitrectomy by proposed means of less surgical risks, less surgeon time, lower costs, and a transition to office-based vitreoretinal procedures. However, there are few data concerning the effect of plasmin at the vitreoretinal interface of human eyes.[4] Especially the impact of plasmin as an enzymatic adjunct to vitrectomy has not been studied and published as yet. Therefore, we compared the ultrastructure of the vitreoretinal interface of human postmortem eyes, which had undergone conventional vitrectomy or plasmin-assisted vitrectomy.

Methods and results

Five human postmortem eyes were injected with one unit (1U) of plasmin (Sigma®, Germany) into the center of the vitreous cavity. The fellow eyes received calcium and magnesium free phosphate buffered saline and served as controls. Eyes were obtained from the local eye bank 6–14 h after death. Due to the lack of blood testing, the corneas were not excised. The donors' age ranged from 55 to 69 years. After 30 min of incubation time at 37°C, all eyes underwent a standard three port pars plana vitrectomy. Induction of PVD was initiated by suction with the vitrectomy probe at the posterior pole and then extended peripherally. The vitreous base was not excised. No attempt of peeling of the posterior hyaloid was made. The globes were placed in a solution of 4% paraformaldehyde, and the vitreoretinal interface was investigated by two observers independently using scanning electron microscopy.

Intraoperatively, all plasmin-treated eyes and three control eyes showed an attached vitreous. In two

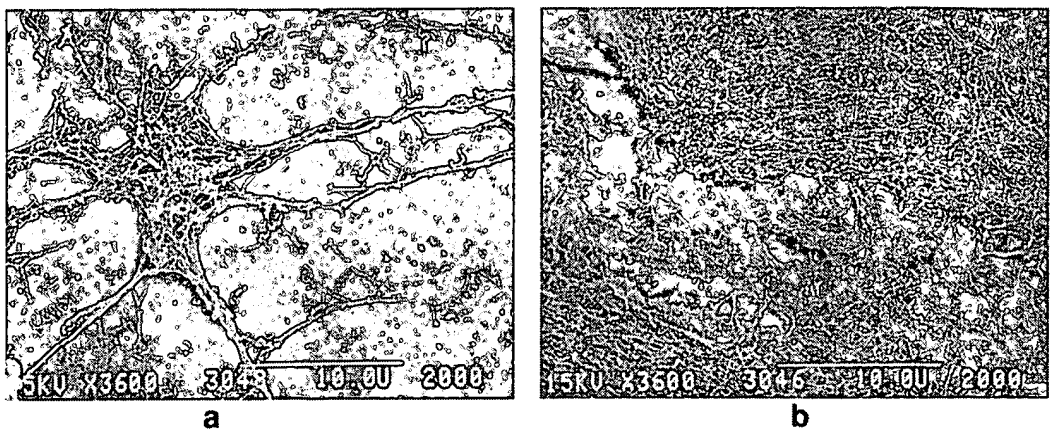

Figure 1 In eyes which had undergone conventional vitrectomy, there were networks of collagen fibrils covering most parts of the ILM.

control eyes, the vitreous was detached spontaneously. However, all control eyes revealed remnants of cortical vitreous at the posterior pole and at the equator (Figure 1). Regarding the networks of collagen fibrils which covered most parts of the ILM, there was no difference in eyes which had undergone spontaneous PVD compared to eyes in which PVD was induced surgically postmortem.

In contrast, plasmin-treated eyes showed only sparse collagen fibrils or a smooth retinal surface (Figure 2). At the vitreous base, there was no vitreoretinal separation.

Comment

This limited series in postmortem human eyes demonstrates the efficacy of plasmin as an enzymatic adjunct to vitrectomy. By eliminating remnants of cortical vitreous, which remain adherent to the ILM following conventional vitrectomy, plasmin-assisted vitrectomy creates a smooth retinal surface consistent with a bare ILM.

It is of note that no attempt of peeling of the cortical hyaloid was made in any eye. The surgical procedure consisted of induction of PVD using the suction forces of the vitrectomy probe over the posterior pole. Maybe the remnants of cortical vitreous could have been removed by meticulous peeling as well. However, every surgical technique approaching the vitreoretinal interface by mechanical means can be technically difficult, and carries the risk of iatrogenic retinal damage.

Regarding the ability of plasmin to create a smooth retinal surface free of cortical vitreous remnants, tractional forces at the vitreoretinal interface may be relieved more safely and effectively compared to conventional vitrectomy. Today, the most complete release of vitreomacular traction seems to be possible by removing all epiretinal tissue including the ILM. ILM peeling has been shown to enhance the closure rate of

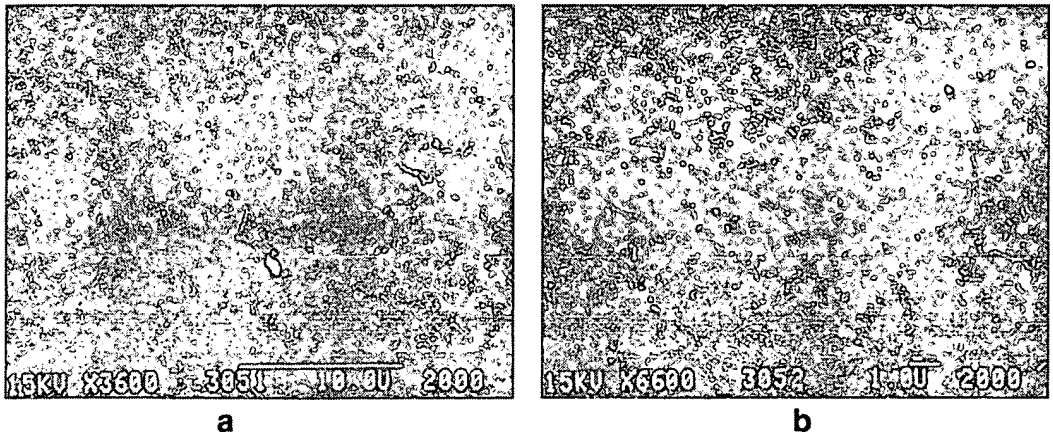

Figure 2 In plasmin-treated eyes, vitrectomy created a smooth retinal surface consistent with a bare ILM.

macular hole surgery and to promote resolution of diffuse diabetic macular edema.[5,6] However, manual peeling of the ILM is technically difficult, and has even been implicated in visual field loss.[7] Staining of the ILM using indocyanine green (ICG) results in better visibility of the membrane, and ICG-assisted peeling has been proposed for an easier and safe removal of the ILM.[8,9] For unknown reasons, however, ICG-assisted peeling of the ILM may cause retinal damage under certain yet undetermined circumstances.[10] Plasmin-assisted vitrectomy may hold the promise of relieving tractional forces without approaching the ILM and the retina.

Indeed, plasmin-assisted vitreous surgery has been proposed as an office-based procedure for idiopathic stage 3 macular holes.[11] Injection of autologous plasmin before vitrectomy was reported to cause PVD and facilitate surgical repair of the hole.[11] Moreover, the procedure has shown promise in the more challenging cases of macular holes caused by ocular trauma.[12] One may speculate whether plasmin could in some cases obviate ILM peeling for complete release of vitreoretinal traction.

In our series we used plasmin as an enzymatic adjunct to vitrectomy. One unit of plasmin applied 30 min before vitrectomy resulted in a smooth retinal surface. Recent studies in postmortem porcine and human eyes demonstrated that enzymatic action alone was sufficient to induce PVD.[2,4] Two units of plasmin created a smooth retinal surface without any additional surgical technique.[2,4] It cannot be concluded from these studies, whether plasmin-assisted vitrectomy presents any advantages compared to plasmin injection alone concerning the cleaving effect and surgical risks.

However, plasmin-induced vitreoretinal separation is limited to the posterior pole and to the equator. At the vitreous base, the cortical hyaloid remains firmly attached, indicating that plasmin does not cleave the vitreoretinal junction by secondary activation of collagenases.[2,4] Therefore, one important disadvantage of plasmin injection without vitrectomy may be the risk of inducing retinal breaks at the posterior margin of the vitreous base. Plasmin assisted vitrectomy enables the surgeon to excise the vitreous base, to examine the peripheral retina for retinal break formation, and to treat retinal breaks immediately.

Further studies are now required to investigate the short- and long-term complications of the different surgical techniques. Before plasmin-assisted vitrectomy may be regarded as a viable alternative or adjunct to vitrectomy, central questions of efficacy and safety need to be addressed. Nevertheless, plasmin-assisted vitrectomy holds the promise of creating a raft of new therapeutic strategies for a variety of vitreoretinal diseases.

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